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労災疾病臨床研究事業

**免疫チェックポイント阻害薬不適応の胸膜中皮腫を対象とした CD26
抗体と PD-1 抗体 Pembrolizumab との併用療法の第 2 相医師主導治験**

令和 7 年度 総括・分担研究報告書

研究代表者 森本 幾夫

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研究代表者 森本 幾夫
順天堂大学大学院医学研究科 免疫病・がん先端治療学講座 特任教授

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研究代表者 森本 幾夫
順天堂大学大学院医学研究科 免疫病・がん先端治療学講座 特任教授

研究分担者 波多野 良
順天堂大学大学院医学研究科 免疫病・がん先端治療学講座 特任准教授

研究分担者 藤本 伸一
岡山労災病院 腫瘍内科 部長・呼吸器内科 部長

研究分担者 上月 稔幸
高知大学医学部 呼吸器・アレルギー内科学 教授

研究分担者 久山 彰一
岩国医療センター 呼吸器内科 臨床研究部長

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研究代表者 森本 幾夫
順天堂大学大学院医学研究科 免疫病・がん先端治療学講座 特任教授

研究分担者 波多野 良
順天堂大学大学院医学研究科 免疫病・がん先端治療学講座 特任准教授

研究分担者 山田 健人
埼玉医科大学医学部 病理学 教授

研究分担者 藤本 伸一
岡山労災病院 腫瘍内科 部長・呼吸器内科 部長

研究協力者 伊藤 匠
順天堂大学大学院医学研究科 免疫病・がん先端治療学講座 特任助教

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I. 総括研究報告

労災疾病臨床研究事業費補助金
総括研究報告書

免疫チェックポイント阻害薬不適応の胸膜中皮腫を対象とした CD26 抗体と PD-1 抗体
Pembrolizumab との併用療法の第 2 相医師主導治験

研究代表者 森本 幾夫 順天堂大学大学院医学研究科
免疫病・がん先端治療学講座 特任教授

研究要旨

悪性胸膜中皮腫はアスベストばく露によって発生する難治性悪性腫瘍である。免疫チェックポイント阻害薬（ICI）が登場して、中皮腫でも PD-1 抗体を軸に化学療法剤などとの併用が新規治療法として認可されたが、全生存期間中央値は約 1.5 年程度にとどまっており、依然として予後は不良である。我々はヒト化 CD26 抗体 YS110 を開発し、フランスにて中皮腫を中心とした第 1 相試験を実施し、国内でも中皮腫に対する第 1/2 相試験を実施した。治療抵抗性の中皮腫患者に対して、CD26 抗体単剤でも高い割合で病態安定（SD）となり中程度の抗腫瘍性は認められたが、有効性がより長時間持続する本抗体を用いた新たな併用療法の開発は重要な課題である。現在実施中の中皮腫の臨床試験は、未治療の患者に対する初回治療、または、化学療法剤不適応例に対する二次治療がほとんどで、ICI 不適応例を対象にした臨床試験はまだ実施されていない。そこで、ICI 抵抗性の切除不能進行胸膜中皮腫に対する CD26 抗体と Pembrolizumab との併用療法の第 2 相試験を計画した。初年度は、治験実施体制の整備、PMDA 対応、治験実施医師・施設の拡充等、臨床試験開始に向けた準備を実施した。

CD26 抗体単剤の国内第 1/2 相試験患者からの検体を用いて、CD26 抗体の有効性が長期間持続する患者を予測できるバイオマーカーの探索を行ってきたが今年度はこれまでに実施した腫瘍病理組織の DNA マイクロアレイデータの解析方法を見直し、DNA マイクロアレイの再解析と定量 PCR による解析を行い、PFS が長期間持続した有効群と無効群とを比較して、有効群に共通して発現が高い遺伝子、無効群に共通して発現が低い遺伝子を同定した。今後、CD26 抗体治療の開始前に抗体療法が有効な患者を予測できるバイオマーカーになり得るかを明らかにする。

研究分担者

波多野 良：順天堂大学大学院医学研究科
免疫病・がん先端治療学講座
特任准教授

藤本 伸一 岡山労災病院 腫瘍内科
部長・呼吸器内科 部長

上月 稔幸 高知大学医学部
呼吸器・アレルギー内科学
教授

久山 彰一 岩国医療センター
呼吸器内科 臨床研究部長

加藤 有加 四国がんセンター
呼吸器内科 医師

山田 健人 埼玉医科大学医学部
病理学 教授

研究協力者

伊藤 匠：順天堂大学大学院医学研究科
免疫病・がん先端治療学講座
特任助教

A. 研究目的

胸膜中皮腫は主にアスベストばく露により発生する胸膜中皮由来の難治性悪性腫瘍である。アスベストばく露から発症までの潜伏期間は約 30-50 年とされ、日本をはじめとしてまだアスベスト使用が禁止されていない中国などアジア・中東では患者数が今後ますます増加すると考えられている。ペメトレキセドとシスプラチンとの化学療法剤の併用が標準治療として用いられてきたが(J Clin Oncol. 2003)、免疫チェックポイント阻害薬(ICI)の登場で、中皮腫でも PD-1 抗体を軸に、化学療法剤などとの併用が新規治療法として認可され、化学療法剤よりも優位性が示唆されている(Lancet. 2021, 2023)。しかし、

全生存期間中央値は 1.5 年以下であり、一定期間は部分奏効(PR)を示すものの病勢進行(PD)となる患者が数多く存在し、これらの患者を救済し得る画期的かつ有効な治療法の開発が切望されている。

研究代表者らは、新規治療標的分子として CD26 に着目し、ヒト化 CD26 抗体を開発してフランスにて中皮腫を中心に First-in-Human 第 1 相臨床試験を行い(Br J Cancer. 2017)、本邦でも治療抵抗性の胸膜中皮腫を対象に第 1 相試験を行い(Lung Cancer. 2019)、抗体推奨用量を 6mg/kg として第 2 相試験を全 31 例に実施した(JTO Clin Res Rep. 2021)。重要なことに、本邦での第 1/2 相試験 計 40 例の中で、ICI で報告されているような重篤な有害事象は少なく、フランス第 1 相試験と同様に良好な安全性が示されている。さらに、本邦での第 1/2 相試験 40 例中 Nivolumab 治療が施行され抵抗性を示した症例が 13 例含まれておりそれらの患者における最良総合効果は、PR が 7.7% (1/13)、病勢安定(SD)が 53.8% (7/13)で、Nivolumab 治療歴のない他の 27 例と同等の有効性を示したことから、CD26 抗体は ICI とは作用機序が異なり、ICI 抵抗性の中皮腫患者にも有効である可能性が強く示唆された。

CD26 抗体単剤での国内第 1/2 相試験では、一部の患者は長期間 PR・SD が持続した(6 か月時点での無増悪生存期間(PFS)率は 25.7% (9/35))だが、PR の割合が低いこと、SD の期間が短い患者がいることから、単剤での効果は中程度であった。

現在も中皮腫に対する様々な臨床試験が行われているが、近年は PD-1 抗体を軸とした 2 剤または多剤併用の治療が主流となっている。そこで、CD26 抗体が ICI とは抗腫

瘍作用機序が異なることに着目し、動物実験で CD26 抗体と PD-1 抗体との併用効果の非臨床 POC を取得した。

- 1) 現在実施中の中皮腫の臨床試験は、未治療の患者に対する初回治療、または、化学療法剤不適応例に対する二次治療がほとんどで、ICI 不適応例を対象にした臨床試験が実施されていない。そこで、上記に示した**現在の中皮腫の標準治療、特に ICI 治療に不適応で有効な治療法がない中皮腫患者**を対象にした、CD26 抗体と PD-1 抗体 Pembrolizumab との併用療法の第2相医師主導治験を計画した。
- 2) さらに CD26 抗体単剤で得られたバイオマーカー検索結果をふまえて CD26 抗体と PD-1 抗体との併用療法の有効性を予測できるバイオマーカーを同定する。本年度は CD26 抗体単剤での有効性を選択できるバイオマーカーを同定する。

B.研究方法

BI. 臨床試験開始準備

1) 治験実施体制の整備・PMDA 対応

令和 7 年度の本研究の研究開始可能日は令和 7 年 9 月 1 日からであったため、まず 9 月から CRO (Contract Research Organization)の選定を行った。CRO 2 社および順天堂大学医学部附属順天堂医院 臨床研究・治験センター (ARO: Academic Research Organization)と委託業務内容の打ち合わせを行い、それぞれの業務の費用と業務間の連携の必要性を考慮して、順天堂大学 ARO と CRO 2 社に委託する各種業務を決定した。また、腫瘍画像評価および中央判定を実施する外部機関の選定も行い、契約締結を行った。統計解析は、利害関係のない大津洋 准教授

(順天堂大学 健康データサイエンス学部)に統計解析責任者を依頼し、解析業務は CRO に委託することにした。

2025 年 10 月 30 日に PMDA との事前面談(相談区分: 新薬審査第五部)を行い、主要評価項目について病勢コントロール率(DCR)から奏効率(ORR)に変更すること、および対面助言相談資料案に記載して欲しい種々の事項の助言を受けた。修正した後相談区分をアカデミアを対象とするレギュラトリーサイエンス戦略相談に変更して追加記入をした後、2025 年 12 月 18 日に 2 回目の事前面談(相談区分: レギュラトリーサイエンス戦略相談)を行った。初回治療に Ipilimumab と Nivolumab の併用治療を行った患者に対する助言を受けた。Pembrolizumab を治験実施計画書案に記載した用法・用量で実施する根拠となる具体的なデータの説明など、対面助言相談資料案に新たに記載して欲しい事項の説明を受け修正した後、PMDA 対面助言に進むよう指示があり 2026 年 1 月に PMDA 対面助言申込みを行い、PMDA から照会事項と機構意見の助言を受け、2026 年 3 月 18 日に PMDA との対面助言を実施した。軽微な修正指示が示されたのみであり、治験実施計画書の内容については概ね合意が得られている。現在、PMDA からの助言への対応を含め、治験実施計画書の細部の修正を行っており、最終化の段階にある。

2) 治験実施医師・施設の拡充

本研究開始時は、藤本伸一先生(岡山ろうさい病院)・久山彰一先生(岩国医療センター)・上月稔幸先生(高知大学医学部附属病院)・加藤有加先生(四国がんセンター)の 4 施設から本試験実施の同意を得られていたが、計画している第 2 相試験の予定症例数が 42

例であることを考慮して、試験期間を短縮するために実施施設をさらに増やすことを検討した。

そこで、関東圏で1施設、北海道で1施設拡充することでより広範囲の中皮腫患者をリクルートできると考え、関東圏、北海道の医療機関として解良恭一先生(埼玉医科大学国際医療センター)および榊原純先生(北海道大学病院)から実施の同意を得た。

BII. バイオマーカー探索の研究手法

分担研究報告書参考

(倫理面への配慮)

本試験については、治験実施計画書を最終化し、患者向け同意説明文書を作成した後、次年度(令和8年度)に、各医療機関の治験審査委員会または中央治験審査委員会において審査を受ける予定である。

なお CD26 抗体単剤の国内第 1/2 相試験患者検体のバイオマーカー探索研究についてはすでに実施承認を得ている。

C. 研究結果

1) CD26 抗体単剤の国内第 1/2 相臨床試験患者の腫瘍病理組織の遺伝子発現解析

本研究の目的は、CD26 抗体療法が有効な患者を選択できるバイオマーカーを探索することである。バイオマーカー探索同意が得られ有効性の評価ができた 22 例を性別・組織型・Nivolumab 投与歴の項目で整理した結果、SD 症例と PD 症例を 3 例以上取れるのは、「男性・上皮型・Nivolumab 投与歴無し」(SD 6 例/PD 4 例)のみであった。そこで DNA マイクロアレイを行う検体として、SD 6 症例中、無増悪生存期間 PFS が長い 3 例(PFS 296 日, 211 日, 139 日)と PD 4 例を選択し

た。これらの SD 3 例の CD26 の発現陽性率は 30%, 70%, 90%で PD 4 例の CD26 の発現陽性率は 95%, 100%, 60%, 95%で腫瘍の CD26 発現陽性率などは CD26 抗体の有効性と関連しないことが示された。

そこで、選択した 7 サンプルそれぞれの腫瘍部位を切り出し、Total RNA 抽出と DNA マイクロアレイ解析を行った。その際、DNA マイクロアレイを実施した 7 サンプルそれぞれで遺伝子によって raw data のシグナル値が極端に低いものが多く見られた。これはホルマリン固定パラフィン包埋組織 (FFPE) から抽出した Total RNA は著しく分解しており、断片化が進んでいるため、DNA マイクロアレイの各遺伝子のプローブ配列の部分の cDNA 増幅合成ができておらず、正確な mRNA の発現量(コピー数)の評価ができていないものと判断した。そこで全遺伝子中、raw data のシグナル値が 10 未満になっているサンプルは no data として扱い、SD は 3 例中 2 例以上、PD は 4 例中 2 例以上 data がある遺伝子だけを解析対象とした。

2) 腫瘍病理組織の DNA マイクロアレイ解析

今回の DNA マイクロアレイを行ったサンプルは、GSEA (Gene Set Enrichment Analysis)解析を最初に行い、特定の生物学的経路や機能に関連する遺伝子群が統計的に有意に変動しているかを明らかにした。登録されている H (Hallmark)のカテゴリの遺伝子セットの中で SD 群と PD 群との間で有意差がある遺伝子セットとして、4 つのセットを見出した。

「TNFA_SIGNALING_VIA_NFKB」、
「EPITHELIAL_MESENCHYMAL_TRANSITION」、
「MTORC1_SIGNALING」の 3 セットでは、SD

群の方がPD群よりも発現が有意に高いことが示された。一方で、「MYOGENESIS」のセットでは、PD群の方がSD群よりも発現が有意に高いことが示された。

さらにそれらの遺伝子セットの中で、SD群の平均発現レベルとPD群の平均発現レベルの差が大きく、かつ群間の標準偏差が小さい遺伝子を選択した。

そこでDNAマイクロアレイに供したサンプルと同様に、増幅合成したcDNAを用いてqRT-PCRを行ったところ、多くの遺伝子でDNAマイクロアレイと共通した傾向が見られた。論文投稿を予定しているため、具体的な遺伝子名は公開しないが、qRT-PCRにおいてもPD症例と比較してSD症例3例に共通して高発現している遺伝子、および、SD症例と比較してPD症例に共通して高発現している遺伝子を見出した。

D. 考察

CD26抗体の有効性予測バイオマーカーを探索するために、CD26抗体単剤の国内第1/2相臨床試験患者の腫瘍病理組織を用いて、SD3例とPD4例のDNAマイクロアレイ解析を行った。

GSEA解析の結果からSD群で発現が高かった遺伝子は、炎症・ストレス応答が持続的に活性化しており、免疫細胞との相互作用が強いことが予想され、また、epithelial mesenchymal transitionが活発で可塑性・浸潤能が高い腫瘍細胞であり、免疫原性が高く免疫療法の効果が出やすい反面、浸潤・転移や免疫抑制環境形成も強い二面性を持つことが示唆される。一方で、PD群で発現が高かった遺伝子は筋細胞、中でも横紋筋に係る遺伝子群が多数見られ、収縮性/筋様

プログラムの増強、細胞外マトリクスの硬化・物理的バリア形成を通じて免疫細胞の浸潤や薬剤到達を阻害している可能性が考えられる。今後は腫瘍病理組織の免疫染色により得られた候補分子の発現を解析する。PFSの期間が長くCD26抗体治療が特に有効な症例とPD症例との判別に有用かどうかを検討する。

E. 結論

CD26抗体単剤の国内臨床試験患者の中皮腫組織から得られたTotal RNAを用いてSD症例3例とPD症例4例のDNAマイクロアレイ解析を行った。解析方法を見直しデータを再解析することで、DNAマイクロアレイの結果と一致してqRT-PCRにおいてもSD症例で共通して発現が高い遺伝子とPD症例で共通して発現が高い遺伝子を見出した。

F. 次年度の予定及び今後の展望

初年度は、治験実施体制の整備、PMDA対応、治験実施医師・施設の拡充等、臨床試験開始に向けた準備を実施した。次年度は、治験実施計画書を最終化し、患者向け同意説明文書および各種手順書を作成した後、治験審査委員会での審査、治験計画届書の提出を行い、第2相試験の開始を予定している。

CD26抗体単剤の国内第1/2相試験患者の腫瘍病理組織を用いて、DNAマイクロアレイの再解析により得られた候補分子の免疫染色を行い、これらの分子がCD26抗体の有効性を予測できるバイオマーカーになり得るかを明らかにするとともにCD26抗体とPembrolizumabとの併用療法においても、同定した遺伝子の発現評価が併用療法の有効性予測にも有用かどうかを検討する。

G. 研究発表

1. 論文発表

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- 2) Zhou Y, Matsumura T, Saito S, Yagi Y, Yoshinaga T, Takeshita M, Fukuda K, Senokuchi T, Motoshima H, Kondo T, Hatano R, Ohnuma K, Morimoto C, Araki E, Kubota N. Hematopoietic dipeptidyl peptidase-4 deficiency promotes M2 macrophage polarization and attenuates atherosclerosis. Biochem Biophys Res Commun. 2026;794:153037.
- 3) Scherpereel A, Baas P, Nowak AK, Tsao AS, Fujimoto N, Peters S, Mansfield AS, Popat S, Bautista Aragon Y, Talbot T, Grossi F, Kowalski D, Kaplan MA, Cardona AF, Soomro R, Hu N, Lee A, Ip V, Hung YH, Zalcman G. Five-Year Clinical Outcomes With Nivolumab Plus Ipilimumab Versus Chemotherapy as First-Line Treatment for Unresectable Pleural Mesothelioma in CheckMate 743. J Clin Oncol. 2026;44(9):742-749.
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2. 著書

なし

3. 学会発表

- 1) 岩尾憲明, 岩澤卓弥, 波多野良, 武田聡, 來栖亜弓, 岡本千香子, 加藤和則, 森本幾夫. 抗CD26抗体は大腸癌細胞の上皮間葉転換を抑制し、転移の進行を阻止する. 第4回日本抗体学会, 2025年12月
- 2) Fujimoto Nobukazu. Mesothelioma Research & Treatment in Japan. International Mesothelioma Interest Group Conference 2025, Plenary Speaker, 2025年10月
- 3) 伊藤匠, 長谷川雄太, 波多野良, 堀本義哉, 佐伯春美, 竹田和由, 江幡正吾, 松岡周二, 大沼圭, 森本幾夫. Type 17 immunity promotes cancer immune evasion through the induction of inflammation. 第84回日本癌学会, 2025年9月

H. 知的財産権の出願・登録状況（予定を含む）

特許取得

- 1) 発明者: 森本幾夫、波多野良、伊藤匠、大沼圭、金子有太郎. 発明の名称: インターロイキン-26 阻害薬を用いたがん治療. 国際出願日: 2026年1月16日, 出願番号: PCT/JP2026/001260, 出願人: ワイズ・エー・シー株式会社、学校法人順天堂

実用新案登録

なし

その他

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

免疫チェックポイント阻害薬抵抗性の切除不能進行胸膜中皮腫を対象とした
CD26 抗体・ペムブロリズマブ併用療法の第 2 相臨床試験の開始準備

研究代表者	森本 幾夫	順天堂大学大学院医学研究科 免疫病・がん先端治療学講座 特任教授
研究分担者	波多野 良	順天堂大学大学院医学研究科 免疫病・がん先端治療学講座 特任准教授
研究分担者	藤本 伸一	岡山労災病院 腫瘍内科 部長・呼吸器内科 部長
研究分担者	上月 稔幸	高知大学医学部 呼吸器・アレルギー内科学 教授
研究分担者	久山 彰一	岩国医療センター 呼吸器内科 臨床研究部長
研究分担者	加藤 有加	四国がんセンター 呼吸器内科 医師

研究要旨

胸膜中皮腫はアスベストばく露によって発生する難治性悪性腫瘍である。免疫チェックポイント阻害薬(ICI)が登場し、中皮腫でも PD-1 抗体を軸に、CTLA-4 抗体や化学療法剤との併用が新規治療法として認可されたが、全生存期間中央値は約 1.5 年程度にとどまっており、依然として予後は不良である。われわれは、新規治療標的分子として胸膜中皮腫に発現する CD26 に着目し、ヒト化 CD26 抗体 YS110 を開発した。フランスにて中皮腫を中心とした第 1 相試験を実施し、国内でも中皮腫に対する第 1/2 相試験を実施した。治療抵抗性の中皮腫患者に対して、CD26 抗体単剤でも高い割合で病勢安定(SD)となり中程度の抗腫瘍効果は認められたが、有効性がより長期間持続する本抗体を用いた新たな併用療法の開発は重要な課題である。また、現在実施中の中皮腫の臨床試験は、未治療の患者に対する初回治療、または、化学療法剤不適応例に対する二次治療がほとんどで、ICI 不適応例を対象にした臨床試験が実施されていない。そこで、ICI 抵抗性の切除不能進行胸膜中皮腫に対する CD26 抗体と Pembrolizumab との併用療法の第 2 相試験を計画した。初年度は、治験実施体制の整備、PMDA 対応、治験実施医師・施設の拡充等、臨床試験開始に向けた準備を実施した。

A. 研究目的

胸膜中皮腫は主にアスベストばく露により発生する胸膜中皮由来の難治性悪性腫瘍である。アスベストばく露から発症までの潜伏期間は約 30-50 年とされ、日本を含め中

国やインドなどアジア・中東では患者数が今後ますます増加すると考えられている。ペメトレキセドとシスプラチンとの化学療法剤の併用が標準治療として長らく選択されてきたが(J Clin Oncol. 2003)、免疫チェック

ポイント阻害薬(ICI)が登場し、中皮腫でも PD-1 抗体を軸に、CTLA-4 抗体や化学療法剤との併用が新規治療法として認可され、特に肉腫様の中皮腫に対して化学療法剤よりも優位性が示唆されている(Lancet. 2021, 2023)。しかしながら、全生存期間中央値は 1.5 年以下であり、一定期間は部分奏効(PR)を示すもののその後病勢進行(PD)となる患者や、治療開始早期に PD を呈する患者が数多存在し、これらの患者を救済し得る画期的かつ有効な治療法の開発が切望されている。

研究代表者らは、新規治療標的分子として中皮腫細胞に発現する CD26 に着目し、ヒト化 CD26 抗体を開発してフランスにて中皮腫を中心に First-in-Human 第 1 相臨床試験を行った(Br J Cancer. 2017)。本邦でも治療抵抗性の胸膜中皮腫を対象に第 1 相試験を 3 用量、各 3 例に(Lung Cancer. 2019)、抗体推奨用量を 6mg/kg として第 2 相試験を全 31 例に実施した(JTO Clin Res Rep. 2021)。重要なことに、本邦での第 1/2 相試験 計 40 例の中で、ICI で報告されているような重篤な有害事象は、第 1 相では 11.1% (1/9)、第 2 相では 19.4% (6/31)と少なく、フランス第 1 相試験と同様に良好な安全性が示されている。さらに、本邦での第 1/2 相試験 40 例中、化学療法後に Nivolumab 治療が施行され、Nivolumab に対して抵抗性を示した症例が 13 例含まれていた。それらの患者における最良総合効果は、PR が 7.7% (1/13)、病勢安定(SD)が 53.8% (7/13)であった。Nivolumab 治療歴のない他の 27 例と同等の有効性を示したことから、CD26 抗体は ICI とは作用機序が異なり、ICI 抵抗性の中皮腫患者にも有効である可能性が強く示唆された。

CD26 抗体単剤での国内第 1/2 相試験では、

化学療法剤や Nivolumab に抵抗性の中皮腫患者 40 例に対して、最良総合効果は PR が 5.0% (2/40)、SD が 52.5% (21/40)であった。一部の患者は長期間 PR・SD が持続した(6 か月時点での無増悪生存期間(PFS)率は 25.7% (9/35))が、PR の割合が低いこと、SD の期間が短い患者がいることから、単剤での効果は中程度であった。

現在も中皮腫に対する様々な臨床試験が行われているが、近年は PD-1 抗体を軸とした 2 剤または多剤併用の治験が主流となっている。そこで、CD26 抗体が ICI とは抗腫瘍作用機序が異なることに着目し、動物実験で CD26 抗体と PD-1 抗体との併用効果の非臨床 POC を取得した(2021-2023 年度労災疾病臨床研究事業費補助金、特許 PCT 出願。2022, 論文作成中)。

現在実施中の中皮腫の臨床試験は、未治療の患者に対する初回治療、または、化学療法剤不適応例に対する二次治療がほとんどで、ICI 不適応例を対象にした臨床試験が実施されていない。そこで、上記に示した**現在の中皮腫の標準治療、特に ICI 治療に不適応で有効な治療法がない中皮腫患者**を対象にした、CD26 抗体と PD-1 抗体 Pembrolizumab との併用療法の第 2 相医師主導治験を計画した。

B. 臨床試験開始準備

1) 治験実施体制の整備

令和 7 年度の本研究の研究開始可能日は令和 7 年 9 月 1 日からであったため、まず 9 月から CRO (Contract Research Organization) の選定を行った。CRO 2 社および順天堂大学医学部附属順天堂医院 臨床研究・治験センター (ARO: Academic Resear

ch Organization)と委託業務内容の打ち合わせ、各種業務の見積もりの相談を行った。CRO 1 社に全ての業務を委託すると大幅に予算を超過する中、それぞれの業務の費用と業務間の連携の必要性を考慮して、順天堂大学 ARO と CRO 2 社に委託する各種業務を決定した。また、腫瘍画像評価および中央判定を実施する外部機関の選定も並行して実施し、契約締結を行った。統計解析は、研究代表者ならびに治験薬提供企業と利害関係のない大津洋 准教授(順天堂大学 健康データサイエンス学部)に統計解析責任者を依頼し、解析業務は CRO に委託することに決定した。

2) PMDA 対応

2025 年 10 月 30 日に PMDA との事前面談(相談区分: 新薬審査第五部)を行い、主要評価項目を病勢コントロール率(DCR)としていたが、奏効率(ORR)に変更するよう助言を受けた。また、期待奏効率と閾値奏効率の設定、有効性の比較対象の設定についても助言を受けた。その他に、対面助言相談資料案に記載して欲しい事項の説明を受けた。指摘があった点を修正した後、相談区分をレギュラトリーサイエンス戦略相談に変更して、修正した治験実施計画書案の確認を再度受けるよう指示があった。

治験実施計画書案の修正と対面助言相談資料案の追加記入をした後、2025 年 12 月 18 日に 2 回目の事前面談(相談区分: レギュラトリーサイエンス戦略相談)を行った。初回治療に Ipilimumab と Nivolumab の併用治療を行った患者は、他の治験対象群と比較して、ペメトレキセド・プラチナ製剤の化学療法の投与歴の有無が異なるため、国内で推

奨される二次治療が変わることを考慮する必要がある、との助言を受けた。また、Pembrolizumab を治験実施計画書案に記載した用法・用量で実施する根拠となる具体的なデータの説明など、対面助言相談資料案に新たに記載して欲しい事項の説明を受けた。指摘があった点を修正した後、PMDA 対面助言に進むよう指示があった。

2026 年 1 月に PMDA 対面助言申込みを行い、PMDA に修正した治験実施計画書案と追加記入した対面助言相談資料案を提出した。その後、PMDA から照会事項と機構意見の助言を受け、2026 年 3 月 18 日に PMDA との対面助言を実施した。PMDA からは軽微な修正指示が示されたのみであり、治験実施計画書の内容については概ね合意が得られている。現在、PMDA からの助言への対応を含め、治験実施計画書の細部の修正を行っており、最終化の段階にある。

3) 治験実施医師・施設の拡充

令和 7 年 9 月の本研究開始時は、藤本伸一先生(岡山労災病院)・久山彰一先生(岩国医療センター)・上月稔幸先生(高知大学医学部附属病院)・加藤有加先生(四国がんセンター)の 4 施設から本試験実施の同意を得られていたが、計画している第 2 相試験の予定症例数が 42 例であることを考慮して、試験期間を短縮するために実施施設をさらに増やすことを検討した。

上記の 4 施設はいずれも中国・四国地方だが、都道府県別にみた中皮腫による死亡数の年次推移 人口動態統計(厚生労働省ホームページ参照)によると、死亡数が特に多いのは大阪府、東京都、兵庫県、神奈川県、4 都道府県で年間 100-160 人であり、次に北

海道、愛知県、埼玉県、福岡県、千葉県が多く 60-100 人であった。そこで、関東圏で 1 施設、北海道で 1 施設拡充することで国内でより広範囲の中皮腫患者をリクルートできると考えた。関東圏、北海道の医療機関の医師に本試験内容の説明と実施協力の相談をし、解良恭一先生(埼玉医科大学国際医療センター)および榊原純先生(北海道大学病院)から実施の同意を得た。

(倫理面への配慮)

本試験については、治験実施計画書を最終化し、患者向け同意説明文書を作成した後、次年度(令和 8 年度)に、各医療機関の治験審査委員会または中央治験審査委員会において審査を受ける予定である。

C. 次年度の予定

初年度は、治験実施体制の整備、PMDA 対応、治験実施医師・施設の拡充等、臨床試験開始に向けた準備を実施した。次年度は、治験実施計画書を最終化し、患者向け同意説明文書および各種手順書を作成した後、治験審査委員会での審査、治験計画届書の提出を行い、第 2 相試験の開始を予定している。

D. 研究発表

1. 論文発表

- 1) Leatham RP, Freibaum JS, Cohen GS, Welsh R, Tang Y, Shirkey MW, Bardhan SB, Hatano R, Morimoto C, Oh BC, Čiháková D, Bromberg JS, Krummey SM. Identification of a highly functional effector CD8⁺ T cell program after transplantation in mice and humans. Am J Transplant. 2026 in press
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Yoshinaga T, Takeshita M, Fukuda K, Senokuchi T, Motoshima H, Kondo T, Hatano R, Ohnuma K, Morimoto C, Araki E, Kubota N. Hematopoietic dipeptidyl peptidase-4 deficiency promotes M2 macrophage polarization and attenuates atherosclerosis. Biochem Biophys Res Commun. 2026;794:153037.

- 3) Scherpereel A, Baas P, Nowak AK, Tsao AS, Fujimoto N, Peters S, Mansfield AS, Popat S, Bautista Aragon Y, Talbot T, Grossi F, Kowalski D, Kaplan MA, Cardona AF, Soomro R, Hu N, Lee A, Ip V, Hung YH, Zalcman G. Five-Year Clinical Outcomes With Nivolumab Plus Ipilimumab Versus Chemotherapy as First-Line Treatment for Unresectable Pleural Mesothelioma in CheckMate 743. J Clin Oncol. 2026;44(9):742-749.
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2. 著書

なし

3. 学会発表

- 1) 岩尾憲明, 岩澤卓弥, 波多野良, 武田聡, 來栖亜弓, 岡本千香子, 加藤和則, 森本幾夫. 抗 CD26 抗体は大腸癌細胞の上皮間葉転換を抑制し、転移の進行を阻止する. 第 4 回日本抗体学会, 2025 年 12 月
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Conference 2025, Plenary Speaker, 2025 年
10 月

- 3) 伊藤匠, 長谷川雄太, 波多野良, 堀本義哉, 佐伯春美, 竹田和由, 江幡正吾, 松岡周二, 大沼圭, 森本幾夫. Type 17 immunity promotes cancer immune evasion through the induction of inflammation. 第 84 回日本癌学会, 2025 年 9 月

E. 知的財産権の出願・登録状況（予定を含む）

1. 特許取得

- 1) 発明者: 森本幾夫、波多野良、伊藤匠、大沼圭、金子有太郎. 発明の名称: インターロイキン-26 阻害薬を用いたがん治療. 国際出願日: 2026 年 1 月 16 日, 出願番号: PCT/JP2026/001260, 出願人: ワイズ・エー・シー株式会社、学校法人順天堂

2. 実用新案登録

なし

3. その他

なし

労災疾病臨床研究事業費補助金

分担研究報告書

ヒト化 CD26 抗体の有効性予測バイオマーカーの探索：
単剤治療国内第 1/2 相臨床試験の腫瘍病理組織の遺伝子発現解析

研究代表者	森本 幾夫	順天堂大学大学院医学研究科 免疫病・がん先端治療学講座 特任教授
研究協力者	波多野 良	順天堂大学大学院医学研究科 免疫病・がん先端治療学講座 特任准教授
研究分担者	山田 健人	埼玉医科大学医学部 病理学 教授
研究分担者	藤本 伸一	岡山労災病院 腫瘍内科 部長・呼吸器内科 部長
研究協力者	伊藤 匠	順天堂大学大学院医学研究科 免疫病・がん先端治療学講座 特任助教

研究要旨

胸膜中皮腫はアスベストばく露によって発生する難治性悪性腫瘍であり、現時点で満足できる治療法はなく、新規治療法の開発が望まれている。われわれは、新規治療標的分子として胸膜中皮腫に発現する CD26 に着目し、ヒト化 CD26 抗体を開発した。フランスにて第 1 相試験を、国内で胸膜中皮腫に対する第 1/2 相試験を実施した結果、治療抵抗性の中皮腫患者に対して CD26 抗体単剤で中程度の効果が認められ、6 か月以上無増悪生存期間 PFS が持続する患者は約 25%であった。そこで、CD26 抗体単剤の国内第 1/2 相試験患者から提供を受けた検体を用いて、CD26 抗体の有効性が長期間持続する患者を予測できるバイオマーカーの探索を行ってきた。今年度はこれまでに実施した腫瘍病理組織の DNA マイクロアレイデータの解析方法を見直し、DNA マイクロアレイの再解析と定量 PCR による解析を行い、PFS が長期間持続した有効群と無効群とを比較して、有効群に共通して発現が高い遺伝子、無効群に共通して発現が低い遺伝子を同定した。今後、中皮腫の病理組織の免疫染色を検討し、CD26 抗体治療の開始前に抗体療法が有効な患者を予測できるバイオマーカーになり得るかを明らかにする。

A. 研究目的

胸膜中皮腫は主にアスベストばく露により発生する難治性悪性腫瘍である。予後は極めて不良であり、診断時には既に進行していることが多く、外科的治療の適応は限られている。さらに、放射線療法や化学療法の有効性も限定的であり、新規治療法の開発が切望

されている。研究代表者らは、新規治療標的分子として中皮腫細胞に発現する CD26 に着目し、ヒト化 CD26 抗体を開発してフランスにて中皮腫を中心に First-in-Human 第 1 相試験を行った(Br J Cancer. 2017)。治療抵抗性の中皮腫患者 22 例に対する最良総合効果は、PR 0 例・SD 10 例・PD 9 例・

評価不能 3 例であった。SD 10 例中、無増悪生存期間 PFS 6 か月以上が 5 例で、最長では PFS が 399 日持続した。この結果を受け、本抗体療法が安全かつ効果的に行われるうえで、CD26 抗体療法の有効性が長期間持続する患者を予測できるバイオマーカーの探索が課題として挙げられた。

本邦で開始した治療抵抗性の胸膜中皮腫を対象とした CD26 抗体単剤の第 1/2 相試験においても、全 40 例の最良総合効果は、PR が 5.0% (2/40)、SD が 52.5% (21/40)であり、治療抵抗性の中皮腫患者に対して中程度の効果は認められた(JTO Clin Res Rep. 2021)。6 か月 PFS 率は 25.7% (9/35)であり、一部の患者には比較的長期間有効性が持続するのに対して、SD の期間が短い患者もいることから、国内の第 1/2 相試験患者の(1)中皮腫病理組織、(2)血清、(3)末梢血リンパ球を用いて、CD26 抗体の有効性予測バイオマーカーの探索に取り組んできた(2021-2023 年度 労災疾病臨床研究事業費補助金 研究報告書に記載)。

(1)中皮腫病理組織を用いた解析には、治療開始前の診断目的で採取された腫瘍組織をホルマリン固定・パラフィン包埋(FFPE)したものを用いているが、FFPE 組織から抽出した Total RNA は著しく分解しており、断片化が進んでいるため、DNA マイクロアレイや qRT-PCR による解析には注意が必要である。そこで、本研究ではこれまでの解析方法を見直し、DNA マイクロアレイデータの再解析と qRT-PCR による解析を行い、CD26 抗体療法の有効性予測バイオマーカーを探索した。

B. 研究方法

1) 腫瘍病理組織

CD26 抗体単剤の胸膜中皮腫に対する国内第 1/2 相臨床試験は第 1 相が全 9 例、第 2 相が全 31 例で行われたが、その中で腫瘍病理組織のバイオマーカー探索に同意が得られたのは、第 1 相が 2 例、第 2 相が 21 例の計 23 例であった。化学療法や抗 PD-1 抗体 Nivolumab 治療前に採取された中皮腫組織をホルマリン固定して作製されたパラフィンブロックの組織切片的提供を受けた。

2) DNA マイクロアレイ解析

中皮腫の FFPE 組織からマイクロダイセクションにより切り出した腫瘍部位を溶解し、miRNeasy FFPE Kit (QIAGEN)を用いて Total RNA 抽出を行った。全 7 サンプルの中で最も得られた RNA 量が少なかったサンプルに合わせて RNA 44ng から TransPlex Whole Transcriptome Amplification Kit (Sigma-Aldrich)と Titanium Taq DNA Polymerase (Clontech)を用いて cDNA の合成と増幅を行った。SureTag DNA Labeling Kit (Agilent Technologies)を用いて cDNA の断片化、DNA の Cy3 標識、精製を行った後、SurePrint G3 Human GE マイクロアレイ 8 x 60K Ver 3.0 (Design ID:072363) (Agilent Technologies)を用いて DNA マイクロアレイ解析を行った。

3) qRT-PCR 解析

中皮腫の FFPE 組織から腫瘍部位を切り出し、miRNeasy FFPE Kit を用いて Total RNA 抽出を行った後、ランダムヘキサマープライマーを用い、PrimeScript II first strand cDNA synthesis kit (TaKaRa Bio)を使用して cDNA を合成した。また、Total

RNA を TransPlex Whole Transcriptome Amplification Kit と Titanium Taq DNA Polymerase を用いて合成・増幅した cDNA でも qRT-PCR を実施した。mRNA の定量は、QuantStudio 6 Pro System および SYBR Green qPCR Master Mix (Thermo Fisher Scientific, Waltham, MA)を用いて行った。得られたデータは、Design & Analysis Software 2.6.0 (Applied Biosystems, Foster City, CA)を用いて解析し、3つの housekeeping 遺伝子の発現量で正規化した。

(倫理面への配慮)

CD26 抗体単剤の国内第 1/2 相試験の患者検体を用いたバイオマーカー探索研究については、各実施施設内の治験審査委員会にて、試験の実施と合わせてバイオマーカー探索用採血・腫瘍組織検体の提供について協議され、実施承認を取得済みである。検体の提供を受ける際には、研究対象者に対する人的擁護上の配慮及び研究により研究対象者が受ける不利益、利益等の説明を行い、書面でのインフォームド・コンセントを得ている。

C. 研究結果

1) CD26 抗体単剤の国内第 1/2 相臨床試験患者の腫瘍病理組織の遺伝子発現解析

本研究の目的は、CD26 抗体療法が有効な患者を選択できるバイオマーカーを探索することである。CD26 抗体単剤の国内第 1/2 相試験で腫瘍病理組織のバイオマーカー解析に同意が得られたのは、第 1/2 相が全 40 例中 23 例で、内訳は PR 0 例・SD 16 例・PD 6 例・評価不能 1 例であった。性別・組織型・Nivolumab 投与歴は CD26 抗体の有

効性に影響する可能性があるため、DNA マイクロアレイ解析で SD 症例と PD 症例の遺伝子発現比較を行う際に、それらの背景を揃える必要がある。バイオマーカー探索同意が得られ有効性の評価ができた 22 例を性別・組織型・Nivolumab 投与歴の項目で整理した結果、SD 症例と PD 症例を 3 例以上取れるのは、「男性・上皮型・Nivolumab 投与歴無し」(SD 6 例/PD 4 例)のみであった。そこで DNA マイクロアレイを行う検体として、SD 6 症例中、無増悪生存期間 PFS が長い 3 例(PFS 296 日, 211 日, 139 日)と PD 4 例(PFS 43 日, 43 日, 43 日, 42 日)を選択した。これらの SD 3 例の CD26 の発現陽性率は 30%, 70%, 90%で H score は 65, 110, 130 であったのに対して、PD 4 例の CD26 の発現陽性率は 95%, 100%, 60%, 95%で H score は 260, 280, 90, 215 であり、腫瘍の CD26 発現陽性率や発現強度は CD26 抗体の有効性と相関しないことが示された。

そこで、選択した 7 サンプルそれぞれの腫瘍部位を切り出し、Total RNA 抽出と DNA マイクロアレイ解析を行った。提供があった組織切片上の腫瘍細胞の面積には個人差があり、また、いずれも FFPE 組織であることから、微量 RNA から合成した cDNA を増幅させる工程を入れた後、DNA マイクロアレイを実施した。SD 群 3 例と PD 群 4 例との間で遺伝子発現の群比較を行い、PD 群と比較して SD 群で高発現している遺伝子と SD 群と比較して PD 群で高発現している遺伝子の抽出を行った。その際、DNA マイクロアレイを実施した 7 サンプルそれぞれで遺伝子によって raw data のシグナル値が極端に低いものが多く見られた。これは FFPE 組織から抽出した Total RNA は

著しく分解しており、断片化が進んでいるため、DNA マイクロアレイの各遺伝子のプローブ配列の部分の cDNA 増幅合成ができておらず、正確な mRNA の発現量(コピー数)の評価ができていないものと判断した。そこで全遺伝子中、raw data のシグナル値が 10 未満になっているサンプルは no data として扱い、SD は 3 例中 2 例以上、PD は 4 例中 2 例以上 data がある遺伝子だけを解析対象とした。

2) 腫瘍病理組織の DNA マイクロアレイ解析および qRT-PCR 解析

今回の DNA マイクロアレイを行ったサンプルは、FFPE 組織由来の分解が著しく、強く断片化した Total RNA で、かつ、微量 RNA であるため増幅合成した cDNA を用いていることから、個々の遺伝子発現差ではなく、GSEA (Gene Set Enrichment Analysis) 解析を最初に行い、特定の生物学的経路や機能に関連する遺伝子群が統計的に有意に変動しているかを明らかにした。登録されている H (Hallmark) のカテゴリーの遺伝子セットの中で SD 群と PD 群との間で有意差がある遺伝子セットとして、4 つのセットを見出した。「TNFA_SIGNALING_VIA_NFKB」、「EPITHELIAL_MESENCHYMAL_TRANSITION」、「MTORC1_SIGNALING」の 3 セットでは、SD 群の方が PD 群よりも発現が有意に高いことが示された。一方で、「MYOGENESIS」のセットでは、PD 群の方が SD 群よりも発現が有意に高いことが示された。

さらにそれらの遺伝子セットの中で、SD 群の平均発現レベルと PD 群の平均発現レベルの差が大きく、かつ群間の標準偏差が小

さい遺伝子を選択した。また、FFPE サンプルから抽出した Total RNA の qRT-PCR では、サンプルによってランダムに断片化しているため、1 遺伝子につき複数の位置でプライマーを設計して検討することが推奨されている (Sci Rep. 2016)。そのため、選択した各遺伝子それぞれに対して 4 つのプライマーを設計し、qRT-PCR を実施した。

まず FFPE 組織から抽出した Total RNA を random hexamer を用いて cDNA に合成したサンプルで qRT-PCR を行ったが、CT 値はいずれのプライマーでも 30 以上であり、増幅工程が入っていない cDNA では PCR による発現評価は難しいことが示唆された。

そこで DNA マイクロアレイに供したサンプルと同様に、増幅合成した cDNA を用いて qRT-PCR を行ったところ、多くの遺伝子で DNA マイクロアレイと共通した傾向が見られた。2026 年度中に論文投稿を予定しているため、具体的な遺伝子名は公開しないが、qRT-PCR においても PD 症例と比較して SD 症例 3 例に共通して高発現している遺伝子、および、SD 症例と比較して PD 症例に共通して高発現している遺伝子を見出した。

D. 考察

CD26 抗体の有効性予測バイオマーカーを探索するために、CD26 抗体単剤の国内第 1/2 相臨床試験患者の腫瘍病理組織を用いて、SD 3 例と PD 4 例の DNA マイクロアレイ解析を行った。

GSEA 解析の結果から SD 群で発現が高かった遺伝子は、炎症・ストレス応答が持続的に活性化しており、免疫細胞との相互作用が強いことが予想され、また、epithelial

mesenchymal transition が活発で可塑性・浸潤能が高い腫瘍細胞であり、免疫原性が高く免疫療法の効果が出やすい反面、浸潤・転移や免疫抑制環境形成も強い二面性を持つことが示唆される。一方で、PD 群で発現が高かった遺伝子は筋細胞、中でも横紋筋に関係する遺伝子群が多数見られ、収縮性/筋様プログラムの増強、細胞外マトリクスの硬化・物理的バリア形成を通じて免疫細胞の浸潤や薬剤到達を阻害している可能性が考えられる。これは腫瘍細胞自身に横紋筋に関係する遺伝子が高発現しているのか、もしくは、組織上で腫瘍細胞に筋細胞も混入している可能性も考えられるが、SD 症例は 3 例ともそれらの発現は高くなかったことから、SD 群と PD 群を判別する組織学上の違いを反映している可能性も考えられる。

医療機関にて組織切片から腫瘍細胞部分を切り出して mRNA レベルで発現評価を行うことは困難であるため、有効性予測バイオマーカー分子は腫瘍組織の免疫染色で評価できることが望ましい。そのため、今後は腫瘍病理組織の免疫染色により得られた候補分子の発現を解析する。PFS の期間が長く CD26 抗体治療が特に有効な症例と PD 症例との判別に有用かどうかを検討する。

E. 結論

CD26 抗体単剤の国内第 1/2 相試験患者の FFPE 中皮腫組織から腫瘍部位をマイクロダイセクションで切り出し、得られた微量の Total RNA を用いて SD 症例 3 例と PD 症例 4 例の DNA マイクロアレイ解析を行った。解析方法を見直し、得られたデータを再解析することで、DNA マイクロアレイの結果と一致して qRT-PCR においても SD 症例で共

通して発現が高い遺伝子と PD 症例で共通して発現が高い遺伝子を見出した。

F. 今後の展望

CD26 抗体単剤の国内第 1/2 相試験患者の腫瘍病理組織を用いて、今回の DNA マイクロアレイの再解析により得られた候補分子の免疫染色を行い、これらの分子が CD26 抗体の有効性を予測できるバイオマーカーになり得るかを明らかにする。

信頼性の高い有効性予測バイオマーカーを同定した後、CD26 抗体と Pembrolizumab との併用療法においても、同定した遺伝子の発現評価が併用療法の有効性予測にも有用かどうかを検討する。

G. 研究発表

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1. 特許取得
- 1) 発明者: 森本幾夫、波多野良、伊藤匠、大沼圭、金子有太郎. 発明の名称: インターロイキン-26阻害薬を用いたがん治療. 国際出願日: 2026年1月16日, 出願番号: PCT/JP2026/001260, 出願人: ワイズ・エー・シー株式会社、学校法人順天堂
2. 実用新案登録
なし
3. その他
なし

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

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
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IV. 研究成果の別刷



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Original Article

Identification of a highly functional effector CD8⁺ T cell program after transplantation in mice and humans

Riley P. Leatham^{1,†} , Joel S. Freibaum^{1,†} , Gregory S. Cohen^{1,†} ,
 Robin Welsh¹ , Yukai Tang¹ , Marina W. Shirkey² , Sanniddhya B. Bardhan¹ ,
 Ryo Hatano³ , Chikao Morimoto³ , Byoung Chol Oh⁴ , Daniela Čiháková^{1,5} ,
 Jonathan S. Bromberg⁶ , Scott M. Krummey^{1,*}

¹ Department of Pathology, Johns Hopkins School of Medicine, Baltimore, Maryland, USA

² Department of Surgery, Center for Vascular and Inflammatory Diseases, University of Maryland School of Medicine, Baltimore, Maryland, USA

³ Department of Therapy Development and Innovation for Immune Disorders and Cancers, Graduate School of Medicine, Juntendo University, Tokyo, Japan

⁴ Department of Plastic and Reconstructive Surgery, Johns Hopkins School of Medicine, Baltimore, Maryland, USA

⁵ W. Harry Feinstone Department of Microbiology and Immunology, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, Maryland, USA

⁶ Departments of Surgery and Microbiology and Immunology, Center for Vascular and Inflammatory Diseases, University of Maryland School of Medicine, Baltimore, Maryland, USA

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ABSTRACT

To better understand the mechanism by which T cells mediate allograft rejection, we investigated the fate and function of graft-specific CD8⁺ T cells expressing the activated isoform of CD43 in mice and humans. Agonism of the CD43 1B11 receptor *in vitro* induced CD8⁺ T cell proliferation in the presence of subthreshold antigen stimulation, and CD43 1B11 agonism *in vivo* overcame costimulation-blockade induced tolerance and enhanced CD8⁺ T cell cytokine production and cytotoxic function. Effector CD43 1B11⁺ CD8⁺ T cells not only expressed high levels of T-bet but also maintained Interleukin-7 receptor subunit alpha (IL-7R[alpha]), and T cell factor 1 (TCF-1) expression at both effector and memory timepoints. In adoptive transfer experiments, CD43 1B11⁺ CD8⁺ T cells were persistent following graft rechallenge and also formed TCF-1⁺IL-7Rα⁺ memory cells. Human CD8⁺ T cells expressing CD43 and the glycosyltransferase GCNT1 were present in rejecting kidney allografts and had high expression of *IFNG*, *ICOS*, and perforins/granzymes. In

Abbreviations: CTLA-4, Cytotoxic T-Lymphocyte Associated Protein 4; CTV, CellTrace Violet; GCNT1, glucosaminyl (N-acetyl) transferase 1; HEV, high endothelial venule; ICOS, Inducible T-cell Costimulator; IFNG, Interferon-gamma; Ig, immunoglobulin; IL-7R[alpha], Interleukin-7 receptor subunit alpha; mAb, monoclonal antibody; PBS, Phosphate buffered saline; TCF-1, T cell factor 1; TCR, T cell receptor; TNF, tumor necrosis factor.

* Corresponding author. Ross Research Building Room 664B, 720 Rutland Avenue, Baltimore, MD 21205, USA. Tel.: +410-502-9719.

E-mail address: skrumme1@jhmi.edu (S.M. Krummey).

[†] These authors contributed equally: Riley P. Leatham, Joel S. Freibaum, and Gregory S. Cohen.

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healthy human donors and transplant candidates, the CD43 1D4 monoclonal antibody clone defined antigen-experienced cytokine-producing CD8⁺ T cells. In sum, these data support an important role for activated CD43⁺ CD8⁺ T cells as potent effectors and point to a potential role for CD43 1B11 signaling in augmenting effector functions in the context of subthreshold antigen or costimulation.

1. Introduction

T cell-mediated allograft rejection is a major contributor to early graft loss.¹⁻³ Multiple studies have highlighted that CD8⁺ T cells, which have potent cytolytic and cytokine-producing functions, mediate rejection in preclinical models and patients.⁴⁻⁸ In the widely held early precursor model of T cell differentiation, CD8⁺ T cells become either short-lived effector cell populations or longer-lived memory precursor populations in the initial cell divisions after antigen priming. Although many features of effector and memory CD8⁺ T cells have been defined by pathogen infection, the overarching model of CD8⁺ T cell differentiation in transplantation is not well understood.⁹⁻¹²

CD43 is a transmembrane sialomucin that is broadly expressed on leukocytes and functions in cellular signaling, adhesion, and trafficking.¹³⁻¹⁶ However, T cell activation elicits the core-2 O-glycosylation of CD43 by the glycosyltransferase GCNT1,^{17,18} which is denoted by the binding of the CD43 1B11 antibody clone. Our group and others have shown that effector and memory CD8⁺ T cells upregulate expression of the activated CD43 receptor.^{13,19,20} We found that after transplantation, CD43 1B11 CD8⁺ T cells were potent effectors that expressed high levels of granzyme B, the cytokines interferon γ /TNF- α , and rapidly infiltrated allografts.¹⁹ In the present study, we further delineated key aspects of the fate and function of this CD8⁺ T cell population in mice and humans.

2. Materials and methods

2.1. Animals

C57BL/6J, C57BL/6J Ly5.2-Cr (CD45.1), C57BL/6-Tg(TcraTcrb)1100Mjb/J (OT-I), and C56BL/6-Tg(CAG-OVAL) 916Jen/J (Act-mOVA) were obtained from The Jackson Laboratory (Bar Harbor, Maine) and used at 6 to 2 weeks of age. Balb/cJ was bred at Johns Hopkins University. This study was conducted in accordance with the Guide for the Care and Use of Laboratory Animals and approved by the Animal Care and Use Committee at Johns Hopkins University.

2.2. Skin transplantation and in vivo treatments

Full-thickness tail and ear skins were transplanted onto the dorsal thorax of recipient mice as previously described.²¹ As indicated, mice were treated with 250 μ g/dose of CTLA-4

immunoglobulin (Ig) (BioXCell) and anti-CD40L (MR1 clone, BioXCell) or 200 μ g/dose CD43 1B11 monoclonal antibody (mAb) (BioLegend) or rat IgG2a (BioXCell) via i.p. injection.

2.3. Heart transplantation and graft-infiltrating T cell isolation

Heterotopic heart allografts were performed as previously described.²² Graft-infiltrating cells were isolated as described using collagenase II (5000 U/mL) and DNase I (500 U/mL) (Worthington Biochemicals) in HBSS (Corning) followed by a GentleMACS Tissue Dissociator (Miltenyi).²³

2.4. Flow cytometric cell sorting and adoptive transfer

Effector CD8⁺ T cells were collected from the spleen and graft-draining lymph nodes of Balb/c skin grafted CD45.1 mice. For adoptive transfer, postgraft CD45.1⁺ CD44^{hi} CD8⁺ T cell populations were sorted based on CD43 1B11 and CD62L expression using a MoFlo XDP instrument. Either $0.9-1.2 \times 10^5$ (graft rechallenge) or $1.5-2 \times 10^5$ (memory) of each purified cell population was transferred via tail vein into C57BL/6J mice.

2.5. Real-time RT-PCR

Heart graft-infiltrating and splenic CD8⁺ T cells were flow cytometrically sorted into CD43 1B11⁺ and CD43 1B11⁻ CD8⁺ T cells. cDNA was synthesized using protocols and primers as described.²³ Data were analyzed using the 2- $\Delta\Delta$ Ct method and normalized to Hprt expression and naïve CD8⁺ T cells.

2.6. In vitro T cell stimulation

Ninety-six-well flat-bottom tissue culture plates were coated with CD3 ϵ , CD28, or CD43 1B11 mAb (BioLegend) at the indicated concentration in 60 μ L of PBS/well for 90 minutes at 37 C. 2×10^5 naïve CD8⁺ T cells (Miltenyi CD8⁺ T Cell Isolation Kit) stained with CellTrace Violet (CTV) (Invitrogen) per well were incubated at 37 C for 4 days in Complete R10 media and analyzed by flow cytometry as described below.

2.7. In vitro cytokine production and cytotoxicity assay

For cytokine production, 5.0×10^5 CD45.1 lymph node cells from day 15 postgraft mice were cocultured with 5.0×10^5 Balb/c splenocytes for 4 hours in complete R10 media at 37 C in the presence of Monensin (BioLegend). Positive control samples

were treated at 2 $\mu\text{L/mL}$ Cell Activation Cocktail with Brefeldin A (BioLegend). For cytotoxicity, purified postgraft day 15 CD45.1 CD8⁺ T cells (CD8⁺ T Cell Isolation Kit, BioLegend) were cocultured with 2.5×10^3 each of Balb/c and C57Bl/6 B cells (CD43 B cell isolation kit, Miltenyi) in complete R10 media at 37 C for 24 hours.

2.8. Immunofluorescence microscopy

Flash-frozen draining lymph nodes were cut into 6 μm sections, fixed in cold 1:1 acetone:methanol for 5 minutes, and washed in PBS. Slides were incubated in primary antibodies and secondary antibodies diluted in PBST (PBS + 0.03% Triton-X-100 + 0.5% BSA) for 1 hour. Slides were imaged using an Accu-Scope EXC-500 fluorescent microscope (Nikon) and analyzed with Volocity software (PerkinElmer). Mean of mean fluorescence intensity was calculated within the demarcated high endothelial venule (HEV) region. Group comparisons were made by taking the sum of HEV area with fluorescence over total HEV area $\times$ mean fluorescence intensity.

2.9. Murine T cell enrichment and flow cytometry

Magnetic bead enrichment for H-2L^d QL9 (QLSPFPFDL, NIH Tetramer Core Facility) or CD45.1 was performed as described.¹⁹ Samples were stained with Live/Dead near-infrared (NIR) Zombie (BioLegend), indicated antibodies, and the Transcription Factor Staining Kit (eBiosciences). Samples were acquired on a Cytex Aurora 4L or 5L.

2.10. Human peripheral blood cell analysis

Healthy donor peripheral blood was collected in Vacutainer tubes containing sodium heparin (BD Biosciences). For cytokine production, 1.0×10^6 peripheral blood mononuclear cell were stimulated with 0.5 $\mu\text{g/mL}$ CD3 ϵ mAb (OKT3; ThermoFisher) and 10 $\mu\text{g/mL}$ GolgiStop (ThermoFisher) in 96-well U-bottom plates in complete RPMI for 20 hours at 37 C. Samples were incubated with supernatant from 1D4 hybridoma for 30 minutes, followed by antimouse IgG PE, and stained with indicated antibodies.

2.11. Human scRNAseq kidney transplant biopsy analysis

Four samples from belatacept and tacrolimus-treated patients (“Bela 1,” “Bela 2,” “Bela 3,” and “Tac 3”) were reanalyzed.²⁴ Gene count matrices were generated using Cell Ranger and analyzed in R (4.3.0) using Seurat (4.3.0.1). Cells with excluded with >200, >2500 gene counts, or >20% mitochondrial DNA. Mature CD8⁺ T cells expressed at least 1 chain of CD3, CD8A, and not expressing CD4. The samples were integrated using the top 2000 genes and 50 dimensions, and clustering analysis was used on the first 12 dimensions and 0.5 resolution. Figures were generated with ggplot2 (3.4.3) and pheatmap (1.0.12).

2.12. Quantification and statistical analysis

2.12.1. Statistical analysis

The frequency Ki67⁺ of T cell populations was calculated as $(\text{Ki67}^+ \text{Marker}^+ / \text{Total Marker}^+) \times 100$. The percent of cytotoxicity (killing) was calculated as $100 - [(\% \text{H-2}^d \text{ target cells with effectors} / \% \text{H-2}^d \text{ target cells with effectors}) / (\% \text{H-2}^d \text{ targets no effectors} / \% \text{H-2}^d \text{ target cells no effectors}) \times 100]$. Statistics were performed using GraphPad Prism 9 or 10.

2.12.2. Study approval

Healthy donors and kidney transplant candidates were isolated following protocols approved by the Johns Hopkins Institutional Review Board.

3. Results

3.1. CD43 1B11 agonism with mAb provides a costimulatory signal to CD8⁺ T cells

Prior studies have reported that the CD43 receptor can provide either costimulatory or inhibitory signals to T cells.^{15,25,26} We stimulated purified CD8⁺ T cells with a range of plate-bound CD3 ϵ and CD28 mAb in the presence or absence of plate-bound CD43 1B11 mAb (Fig. 1A). We found that a concentration of 2.5 $\mu\text{g/mL}$ either CD3 ϵ or CD28 mAb elicited uniform proliferation of CD8⁺ T cells (Fig. 1A), whereas either 2.5 $\mu\text{g/mL}$ or 0.625 $\mu\text{g/mL}$ of CD3 ϵ with 0.625 $\mu\text{g/mL}$ CD28 mAb was below a threshold necessary to induce maximal CD8⁺ T cell proliferation (Fig. 1B). However, the addition of CD43 1B11 mAb agonism to these subthreshold stimulation conditions elicited robust proliferation of CD8⁺ T cells (Fig. 1B). These data demonstrate that the CD43 1B11 pathway can provide a costimulatory signal to CD8⁺ T cells when a subthreshold strength of antigen or CD28 stimulation is present.

3.2. CD43 mAb treatment overcomes tolerance to induce graft rejection

We reasoned that because CD43 1B11 can provide activating signals to CD8⁺ T cells and CD43 1B11⁺ CD8⁺ T cells are potent effectors, signaling through this receptor could elicit graft rejection. We induced tolerance with combined CD28/CD40 costimulation blockade (CTLA-4 Ig and CD40L mAb; Fig. 1C) and treated mice with CD43 1B11 mAb on days 8 to 14, which corresponds to the initial expression of CD43 1B11 epitope on CD8⁺ T cells.¹⁹ We found that while costimulation-blockade induced graft survival to 30 days, treatment with CD43 1B11 elicited graft rejection in 73% of mice by day 20 (Fig. 1D). Although costimulation blockade elicited few activated effector CD8⁺ T cells, mice treated with CD43 1B11 had a higher frequency of highly proliferative CD62L^{lo}CD27^{lo} T cell population (Fig. 1E and Supplementary Fig. S1A). CD43 1B11 mAb enhanced the frequency of interferon γ -positive among PD-1⁺ CD8⁺ T cells (Fig. 1F and Supplementary Fig. S1B) and enhanced the cytotoxicity of CD8⁺ T cells against Balb/c target cells (Fig. 1G). Together, these data demonstrate that in the context of limited antigen or

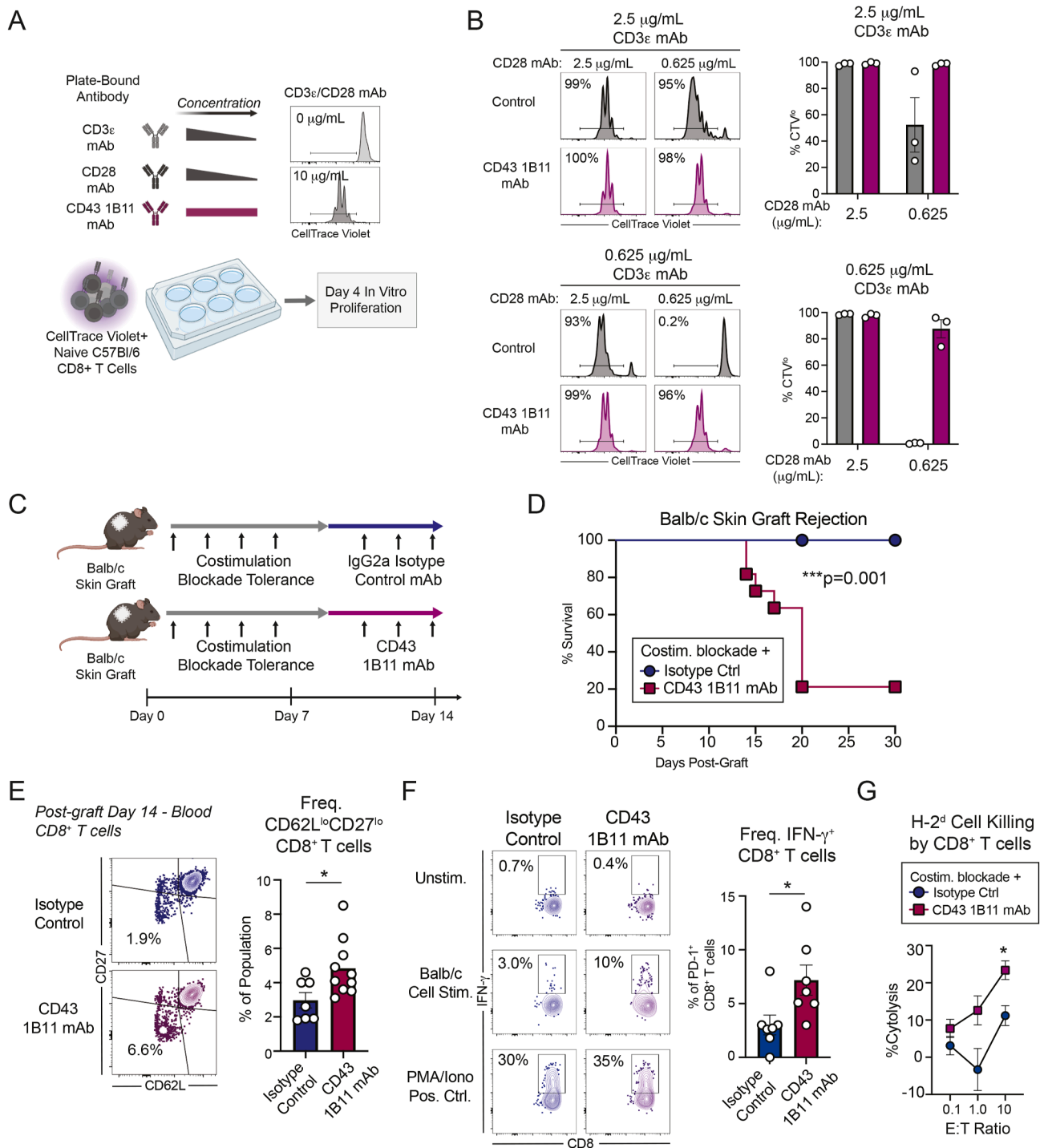


Figure 1. CD43 1B11 agonism of CD8⁺ T cells can overcome subthreshold antigen and costimulation signals. (A) Purified naïve C57Bl/6 CD8⁺ T cells were labeled with CellTrace Violet and incubated in wells coated with varied concentrations of CD3ε, CD28, and CD43 1B11 mAb for 4 days. (B) Example histograms and summary data of CD8⁺ T cells after 4 days of stimulation with the indicated mAb. (C) C57Bl/6 mice were provided with Balb/c skin grafts and treated with tolerance-inducing costimulation blockade with CTLA-4 Ig and CD40L mAb (250 μg/dose, postgraft days 0, 2, 4, and 6). Groups of mice were treated with IgG2a isotype control or CD43 1B11 mAb (200 μg/dose) on postgraft days 8, 11, and 14. (D) Skin graft survival was assessed on mice treated as described in panel A. (E) On postgraft day 14, the phenotype of CD8⁺ T cells was assessed in the blood of skin-grafted mice. (F) Draining lymph node CD8⁺ T cells were restimulated for 4 hours with Balb/c splenocytes to assess for interferon γ production. (G) Purified CD8⁺ T cells were incubated with Balb/c or control C57Bl/6 B cells for 20 hours, and the percent of killed target cells was calculated. Each data point depicts an individual mouse. Summary data depict pooled results from (B) 3 independent experiments ($n = 3$ mice/group), (D) 3 independent experiments ($n = 9 = 11$ /group), (E-G) 2 independent experiments ($n = 5-9$ mice/group). Error bars depict mean $\pm$ SEM. Statistics performed by (B, E, F) unpaired Student's t test (2-tailed), (G) 2-way ANOVA with Sidak's multiple comparisons test. Error bars depict SEM. ANOVA, analysis of variance; CTLA-4, Cytotoxic T-Lymphocyte Associated Protein 4; E:T, effector to target ratio; Ig, immunoglobulin; mAb, monoclonal antibody; N.D., no data; PMA/Iono, phorbol 12-myristate 13-acetate/ionomycin; SEM, standard error of the mean. ** $P < .01$, *** $P < .001$, **** $P < .0001$.

costimulation, CD43⁺ 1B11 agonism can enhance CD8⁺ T cell effector functions and elicit allograft rejection.

3.3. The CD43⁺ ICOS⁺ phenotype specifically identifies a population of proliferative graft-specific CD8⁺ T cells

As transplantation elicits relatively weak expansion of graft-specific CD8⁺ T cells,^{19,27} we next evaluated whether the CD43⁺ 1B11⁺ ICOS⁺ phenotype can be used to identify relatively rare proliferative CD8⁺ T cells after transplantation by evaluating expression of Ki67, which denotes recently divided or actively proliferating T cells, in H-2^b C57Bl/6 mice that received an H-2^d Balb/c skin graft on day 14 postgrafting (Fig. 2A).

Among the canonical activation markers CD44^{hi} or CD62L^{lo}, many CD44^{hi} or CD62L^{lo} cells were Ki67⁺, indicating that these markers are not specific for proliferative graft-specific CD8⁺ T cells (Fig. 2B). Consistent with prior studies,^{19,27} we found that very few KLRG-1⁺ cells were elicited during acute rejection (Fig. 2B), precluding further analysis of KLRG-1⁺ Ki67⁺ cells. We found that 57.9 ± 4.7% and 32 ± 4.3% of CXCR3⁺ cells were Ki67⁺ in the L^d QL9⁺ and bulk CD8⁺ T cell compartments, respectively (Fig. 2B). However, both CD43⁺ 1B11⁺ and ICOS⁺ populations were highly enriched for Ki67⁺ CD8⁺ T cells (Fig. 2B). Among ICOS⁺, 81.1 ± 4.1% of L^d QL9⁺ and 66.0 ± 3.8% of bulk CD8⁺ T cells were Ki67⁺; among CD43⁺ 1B11⁺ cells, 87.5 ± 2.9% L^d QL9⁺ and 64.5 ± 4.7% bulk CD8⁺ T cells were Ki67⁺.

To directly visualize the proliferation of CD8⁺ T cells during acute rejection, we adoptively transferred polyclonal CTV-labeled naïve CD45.1 CD8⁺ T cells into naïve C57Bl/6 (CD45.2) hosts, followed by Balb/c skin grafting (Fig. 2C, D). In parallel, we transferred CTV-labeled Thy1.1⁺ OT-I CD8⁺ T cells into C57Bl/6 hosts and provided Act-mOVA skin grafts as a comparison high-affinity antigen-specific response. After 14 days, we identified the graft-specific L^d QL9⁺ CD8⁺ T cells, bulk CD8⁺ T cells, or OT-I cells using magnetic column enrichment. Expression of ICOS and CD43 1B11 was largely restricted to divided cells in each population (Fig. 2E, F). Among divided bulk CD8⁺ T cells, the frequency of ICOS⁺ cells was 41.6 ± 4.9%, whereas 73.6 ± 4.8% were CD43 1B11⁺. L^d QL9⁺ CD8⁺ T cells displayed a similar profile, with divided cells being 84.2 ± 7.5% ICOS⁺ and 91.8 ± 4.6% CD43 1B11⁺. Thus, at this timepoint, although both ICOS and CD43 1B11 are specific for proliferating CD8⁺ T cells, CD43 1B11 is a modestly more sensitive marker for proliferating graft-specific CD8⁺ T cells.

We also evaluated whether the expression of CD43 1B11 is solely a consequence of strong T cell receptor (TCR) affinity. Among monoclonal OT-I T cells activated with Act-mOVA skin grafts on day 14 (Fig. 2G), a fraction of CD44^{hi} cells expressed CD43 1B11 in the draining lymph nodes (64 ± 11.7%) and spleen (42.4 ± 9.4%, Fig. 2H), strongly suggesting that CD43 1B11 expression is controlled by factors beyond TCR affinity.

3.4. CD43⁺ 1B11⁺ CD8⁺ T cells rapidly infiltrate heart allografts during acute rejection

We next evaluated whether CD43⁺ 1B11⁺ CD8⁺ T cells contributed to acute rejection in fully vascularized heart

allografts. We assessed the phenotype of heart graft-infiltrating CD8⁺ T cells relative to the spleen on day 5 postgraft (Fig. 3A), which corresponds to the peak of acute rejection,^{28,29} and found that although only 7.4 ± 3.6% of CD8⁺ T cells were CD43⁺ 1B11⁺ ICOS⁺ in the spleen at this early timepoint, 53.4 ± 14.7% of graft-infiltrating CD8⁺ T cells were CD43⁺ 1B11⁺ ICOS⁺ (Fig. 3B). Relative to CD43⁺ 1B11⁺ CD8⁺ T cells, graft-infiltrating CD43⁺ 1B11⁺ CD8⁺ T cells expressed similar levels of interferon γ , but elevated levels of granzyme B and granzyme K (Fig. 3C). These data demonstrate that CD43⁺ 1B11⁺ ICOS⁺ comprise a significant portion of the effector graft-infiltrating CD8⁺ T cells during acute rejection.

3.5. CD43⁺ 1B11⁺ CD8⁺ T cells maintain expression of IL-7R α and TCF1 during acute rejection

We next evaluated the expression of key effector and stem-like markers of graft-specific CD44^{hi} CD8⁺ T cells at effector and memory timepoints (Fig. 4A). CD43⁺ 1B11⁺ CD8⁺ T cells were nearly universally positive for Ki67 (Fig. 4B, C). However, CD43⁺ 1B11⁺ CD8⁺ T cells also expressed similarly high levels of IL-7R α to naïve, CD62L^{hi} and CD62L^{lo} effector populations (Fig. 4B, C). CD43⁺ 1B11⁺ effectors expressed significantly higher levels of T-bet than either CD43⁺ 1B11⁺ effector population (85 ± 2.9% CD43⁺ 1B11⁺ vs 48 ± 3.1% CD62L⁺ CD43⁺ 1B11⁺ and 52 ± 5.1% CD62L⁺ CD43⁺ 1B11⁺; Fig. 4B, C). Finally, CD43⁺ 1B11⁺ CD8⁺ T cells also maintained relatively high levels of TCF-1 (69.9 ± 4.3%) similar to naïve and CD43⁺ 1B11⁺ effector CD8⁺ T cell populations (Fig. 4B, C). A similar expression profile of Ki67, IL-7R α , T-bet, and TCF-1 was observed for bulk CD44^{hi} CD8⁺ T cell populations (Supplementary Fig. S2).

At a memory timepoint (>35 days postgraft), we found that all 3 populations of CD8⁺ T cells were high for IL-7R α and TCF-1 (Fig. 4D, E). However, CD43⁺ 1B11⁺ CD8⁺ T cells maintained higher expression of T-bet (72.6 ± 3.9% CD43⁺ 1B11⁺ vs 48.0 ± 5.0% CD62L⁺ CD43⁺ 1B11⁺ and 47.2 ± 6.2% CD62L⁺ CD43⁺ 1B11⁺; Fig. 4D, E). Together, these data show that CD43⁺ 1B11⁺ CD8⁺ T cells not only have an effector and memory program characterized by high T-bet expression vs CD43⁺ 1B11⁺ populations but also largely maintain expression of self-renewal markers IL-7R α and TCF-1.

3.6. Effector CD43⁺ 1B11⁺ CD8⁺ T cells robustly respond after graft rechallenge

We used an adoptive transfer approach to assess the fate of CD43⁺ 1B11⁺ CD8⁺ T cells relative to CD43⁺ 1B11⁺ CD8⁺ T cells. We adoptively transferred equal numbers of effector CD45.1 CD44^{hi} CD62L^{lo} CD43⁺ 1B11⁺ and CD43⁺ 1B11⁺ CD8⁺ T cells into CD45.2 C57Bl/6 mice, challenged them with a Balb/c skin graft, and evaluated the number and phenotype of transferred populations on day 21 postgraft (Fig. 5A). We found that relative to CD43⁺ 1B11⁺ T cells, greater numbers of CD43⁺ 1B11⁺ T cells were recovered from secondary lymphoid tissue (Fig. 5B). Although CD43⁺ 1B11⁺ T cells remained CD43⁺ 1B11⁺ after skin graft rechallenge, a fraction of CD43⁺ 1B11⁺ T cells converted to CD43⁺ 1B11⁺ T cells (Fig. 5C). Evaluation of the localization of

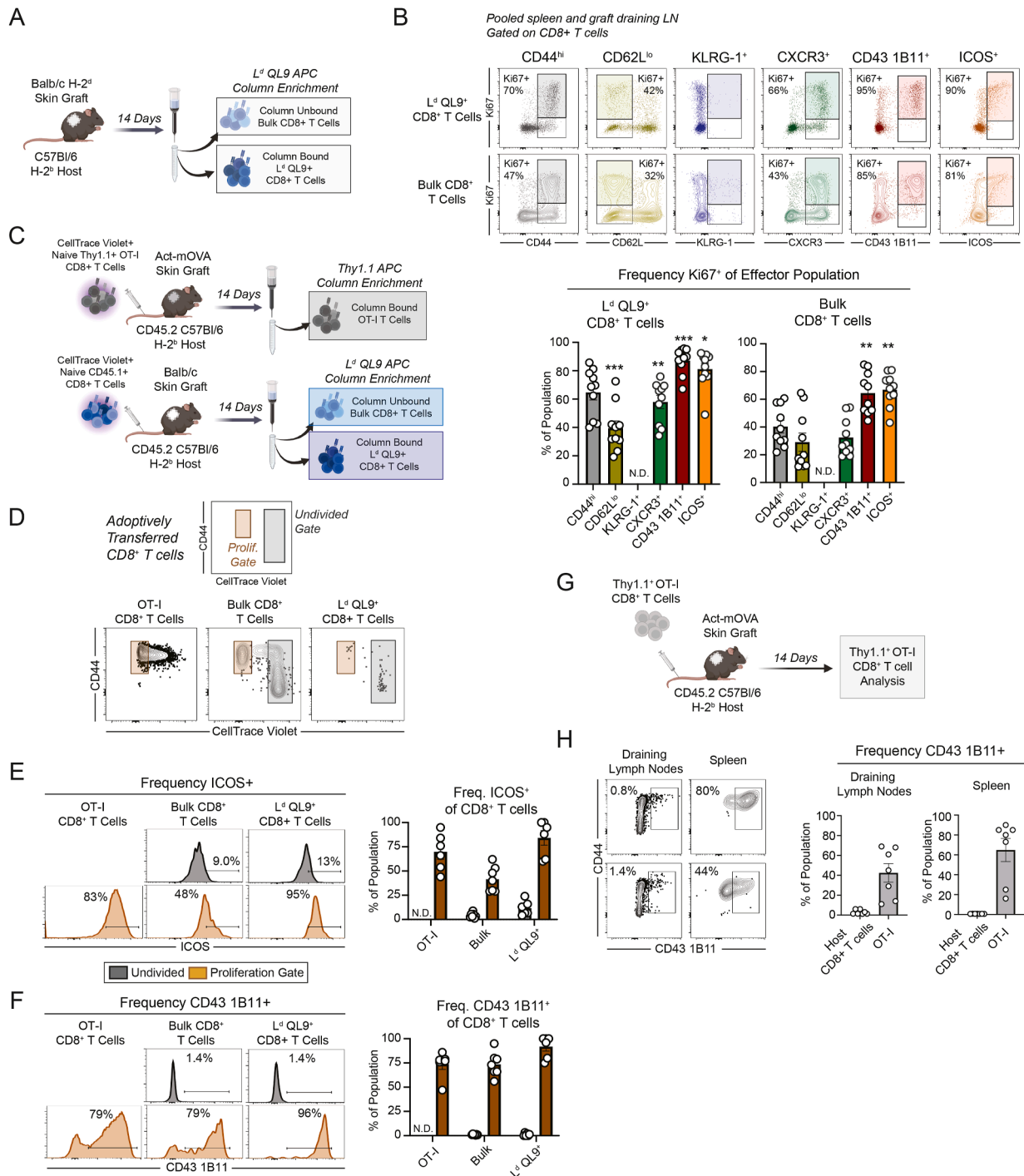


Figure 2. Proliferative CD8⁺ T cells are defined by CD43 1B11⁺ and ICOS⁺ phenotype after transplantation. (A) C57Bl/6 mice were provided with Balb/c skin grafts, and CD8⁺ T cells were assessed on day postgraft 14 in the spleen and graft-draining lymph nodes. (B) Representative flow and summary data depicting the frequency of Ki67⁺ cells among CD8⁺ T cells expressing the indicated marker. Minimal expression of KLRG-1 precluded analysis. (C) 2×10^6 naïve CD45.1 or 1×10^6 Thy1.1 OT-I CD8⁺ T cells labeled with CellTrace Violet were adoptively transferred into CD45.2 C57Bl/6 mice, who were provided with Balb/c or Act-mOVA skin grafts, respectively. Transferred cells were enriched using paramagnetic beads specific for APC, which were stained with Thy1.1 or L^d QL9 tetramers. (D) Among the adoptively transferred CD8⁺ T cell populations, gates were drawn around the undivided (gray) and proliferation (orange) populations. The minimal number of undivided OT-I cells precluded further analysis. (E) The frequency of ICOS⁺ cells among each indicated adoptively transferred CD8⁺ T cell population. (F) Frequency of CD43 1B11⁺ cells among each indicated adoptively transferred CD8⁺ T cell population. (G) C57Bl/6 mice were adoptively transferred with 5×10^5 Thy1.1⁺ OT-I T cells and provided with Act-mOVA skin grafts. (H) The frequency of CD43 1B11⁺ CD8⁺ T cells was assessed in the draining lymph nodes and the spleen. Each data point depicts an individual mouse. Summary data depicts pooled results from 2 to 5 independent experiments ($n = 5-10$ mice/group) and depicts mean $\pm$ SEM. Statistics performed by (B) 1-way ANOVA with Dunnett's multiple comparison test vs CD44^{hi}. Error bars depict SEM. ANOVA, analysis of variance; APC, allophycocyanin; N.D., no data; due to low cell numbers; SEM, standard error of the mean. * $P < .05$, ** $P < .01$, *** $P < .001$.

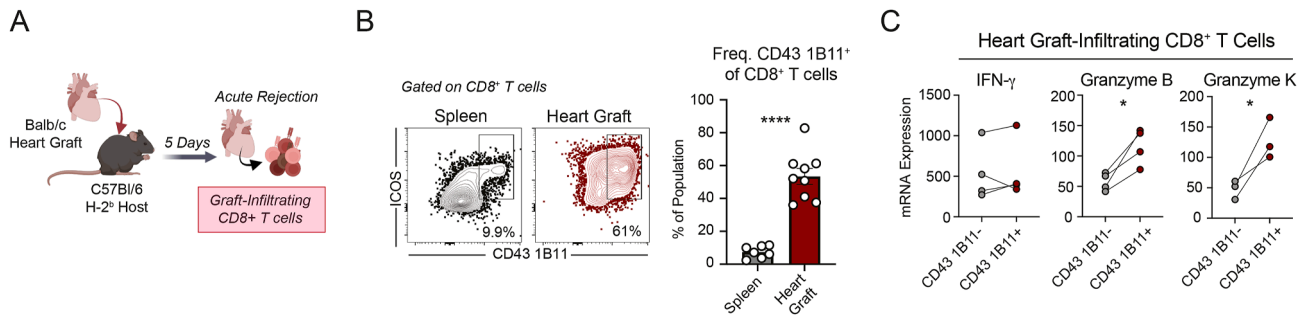


Figure 3. Effector CD43 1B11⁺ CD8⁺ T cells participate in heart allograft rejection. (A) C57Bl/6 mice were provided with heterotopic Balb/c heart grafts and assessed on days 5 to 6 postgraft. (B) The phenotype of CD8⁺ T cells was assessed in the spleen and heart graft on day 5 postgraft. (C) CD43 1B11⁺ and CD43 1B11⁺ CD8⁺ T cells were flow cytometrically sorted from heart grafts, and gene expression was assessed using real-time RT-PCR. Each data point depicts an individual mouse. Summary data depicts pooled results from 2 to 5 independent experiments (n = 5–10 mice/group) and depicts mean ± SEM. Statistics performed by (B) unpaired Student's *t* test (2-tailed) or (B) paired Student's *t* test (2-tailed). Error bars depict SEM. ANOVA, analysis of variance; N.D., no data, due to low cell numbers; SEM, standard error of the mean. **P* < .05, *****P* < .0001.

these transferred populations in the draining lymph node revealed that the CD43 1B11⁺ population localizes to the HEV relative to the CD43 1B11[−] CD8⁺ T cells (Fig. 5D). Together, these data demonstrate that CD43 1B11⁺ CD8⁺ T cells appear to be a terminal phenotype, as they are formed from CD43 1B11[−] precursors, and are persistent after rechallenge *in vivo*.

3.7. Effector CD43 1B11⁺ CD8⁺ T cells are maintained in a memory state in the absence of alloantigen

We next questioned whether effector CD43 1B11⁺ CD8⁺ T cells are short-lived in the absence of alloantigen. We flow cytometrically sorted CD8⁺ CD44^{hi} effectors into CD62L^{hi}, CD43 1B11[−], and CD43 1B11⁺ populations and parked them in congenic C57Bl/6 mice for 35 days (Fig. 6A). We found that CD62L⁺ effectors were recovered at the greatest number, whereas fewer CD43 1B11[−] and CD43 1B11⁺ effectors were recovered in the secondary lymphoid tissue (Fig. 6B).

We next evaluated the phenotype of these transferred memory populations. A fraction of CD62L^{hi} effectors became CD62L^{lo} memory cells, but all remained CD43 1B11[−] (Fig. 6C, D). A significant subset, 30.3 ± 5.5%, of CD43 1B11[−] effectors differentiated into CD43 1B11⁺ memory cells. The majority, 76.9 ± 3.5%, of effector CD43 1B11⁺ cells remained CD43 1B11⁺ memory cells, with a small fraction, 10.27 ± 2.3% becoming CD43 1B11[−] (Fig. 6C). Among the transferred populations, there was a similar high expression of TCF-1 and T-bet among memory subsets (Fig. 6E, F). However, the CD43 1B11⁺ transferred effector population expressed significantly higher levels of IL-7Rα than CD43 1B11[−] transferred effectors (Fig. 6E, F). Together, these results show that effector CD43 1B11⁺ CD8⁺ T cells persist as memory T cells in the absence of alloantigen.

3.8. CD8⁺ T cells that are SPN⁺GCNT1⁺ are present in the rejecting allografts of kidney transplant patients experiencing acute rejection

In order to investigate whether activated CD43⁺ CD8⁺ T cells infiltrate kidney allografts during acute rejection using a data set

published by Shi et al.²⁴ (Supplementary Fig. S3A). Among 4 kidney transplant patients experiencing acute cellular rejection, we identified 8 clusters of CD8⁺ T cells (Supplementary Fig. S3B, C). All clusters expressed the CD43 gene *SPN*, consistent with its uniform expression on most leukocyte populations. We reasoned that expression of the glycosyltransferase GCNT1, which is responsible for glycosylating the CD43 receptor,^{12,17,18} could be a surrogate for CD43 1B11⁺ CD8⁺ T cells. We found that clusters 1, 2, 4, and 6 were SPN⁺GCNT1⁺ (Supplementary Fig. S3D). Analogously to CD43 1B11⁺ CD8⁺ T cells, the SPN⁺GCNT1⁺ clusters expressed relatively higher levels of granzyme B (GZB) and lower levels of CD62L (*SELL*). The SPN⁺GCNT1⁺ clusters also expressed high levels of granzymes and perforin (*GZMA*, *GZMH*, *GZMK*, *PRF1*). Overall, these results reveal that populations of effector SPN⁺GCNT1⁺ CD8⁺ T cells are present in rejecting kidney allografts.

3.9. CD43 1D4 mAb identifies a population of effector CD8⁺ T in healthy human donors and kidney transplant candidates

In humans, the CD43 1D4 mAb clone identifies a large core-2 O-glycosylated isoform of CD43 on human T cells.³⁰ In healthy donors, we found that a fraction of CD14⁺ monocytes and CD8⁺ T cells stained for CD43 1D4 (Fig. 7A). We investigated whether the CD43 1D4⁺ CD8⁺ T cells corresponded with the canonical naïve (CCR7⁺CD45RA^{hi}) or antigen-experienced T cell populations: central memory (T_{CM}, CCR7⁺CD45RA^{lo}), effector memory (T_{EM}, CCR7[−]CD45RA^{lo}), or T effector memory RA⁺ (T_{EMRA}, CCR7[−]CD45RA⁺) populations. Although CD43 1D4[−] CD8⁺ T cells were predominantly found within the naïve compartment, CD43 1D4⁺ CD8⁺ T cells were found predominantly within antigen-experienced fractions (Fig. 7B). Further exploration of the phenotype of these cells revealed that CD43 1D4⁺ CD8⁺ T cells had lower expression of CD27 and higher expression of CXCR3 and ICOS than CD43 1D4[−] CD8⁺ T cells (Fig. 7C).

We next investigated whether CD43 1D4 was expressed on CD8⁺ T cells from patients with end-stage renal disease who are

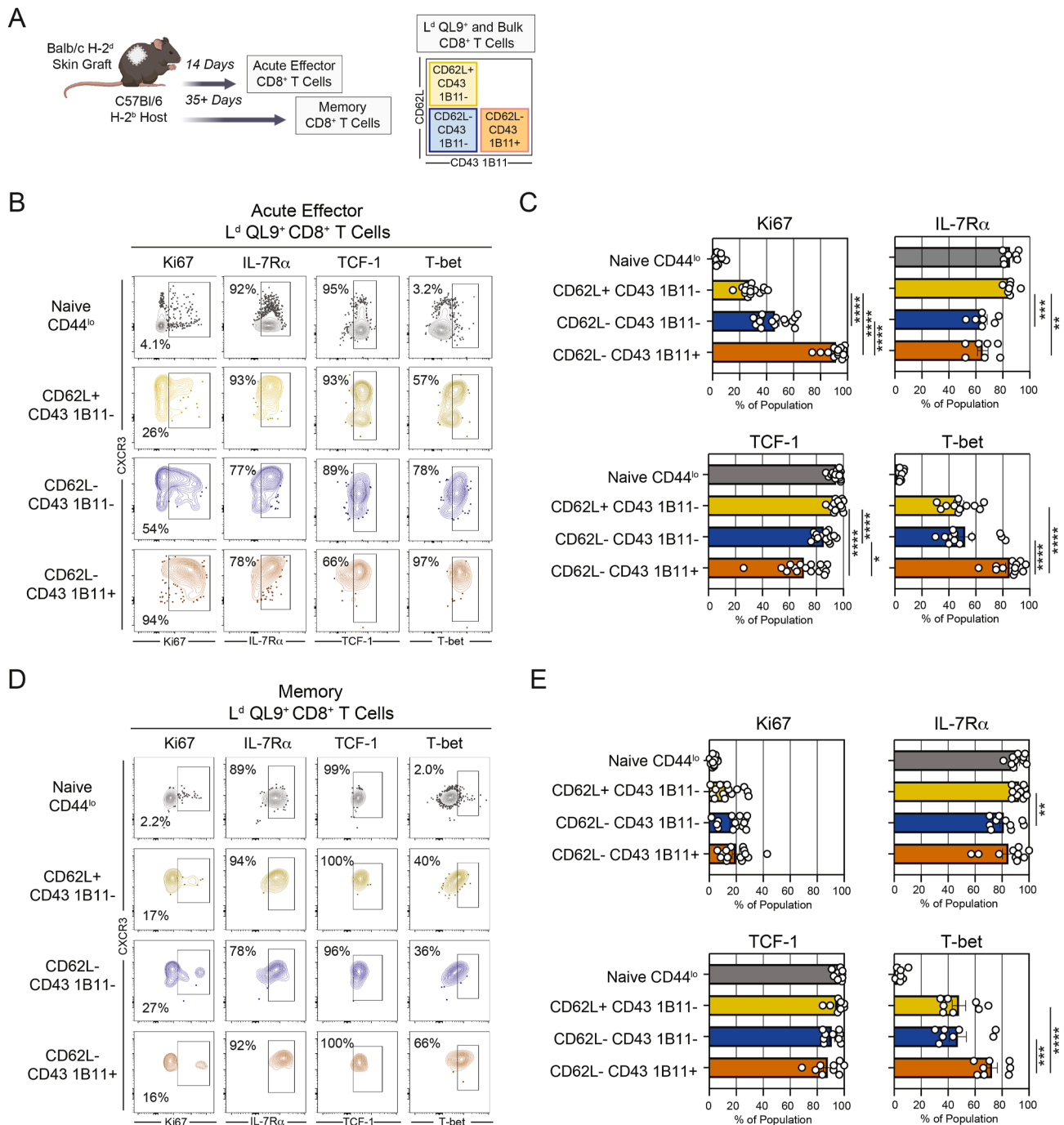


Figure 4. Graft-specific CD43 1B11⁺ CD8⁺ T cells have both effector and stem-like phenotypic features. (A) C57Bl/6 mice were provided with Balb/c skin grafts, and CD8⁺ T cells were assessed on day 14 postgraft (acute effector) or day >35 postgraft (memory) in pooled spleen and graft-draining lymph nodes. Graft-specific cells were identified after enrichment using L^d QL9 tetramers and APC microbeads and gated based on CD62L and CD43 1B11 expression as depicted. (B) Representative flow plots and (C) summary data depicting expression of Ki67, IL-7R α , TCF-1, and T-bet among CD44^{lo} or CD44^{hi} L^d QL9⁺ CD8⁺ T cell populations at the acute effector timepoint. See also [Supplementary Figure S1](#). (D) Representative flow plots and (E) summary data depicting expression of Ki67, IL-7R α , TCF-1, and T-bet among CD44^{lo} or CD44^{hi} L^d QL9⁺ CD8⁺ T cell populations at the memory timepoint. Summary data depicts pooled results from 2 to 3 independent experiments ($n = 8-14$ mice/group) and depicts mean $\pm$ SEM. Statistics performed by (C, E) 1-way ANOVA with Tukey's multiple comparison test (excluding CD44^{lo} population used as a staining control). Error bars depict SEM. ANOVA, analysis of variance; APC, allophycocyanin; IL-7R[α], Interleukin-7 receptor subunit alpha; SEM, standard error of the mean; TCF-1, T cell factor 1. * $P < .05$, ** $P < .01$, *** $P < .001$, **** $P < .0001$.

waitlisted for kidney transplantation ([Supplementary Table](#)). Similar to healthy donors, a population of CD8⁺ T cells expressed CD43 1D4 (Fig. 7D). We found that relative to CD43 1D4⁻, CD43 1D4⁺ CD8⁺ T cells had similar expression of

CD45RA and CCR7, demonstrating that this population did not correspond to a canonical T_{EM}, T_{CM}, or T_{EMRA} populations (Fig. 7E). CD43 1D4⁺ CD8⁺ T cells from these patients also displayed lower expression of CD27 and higher expression of

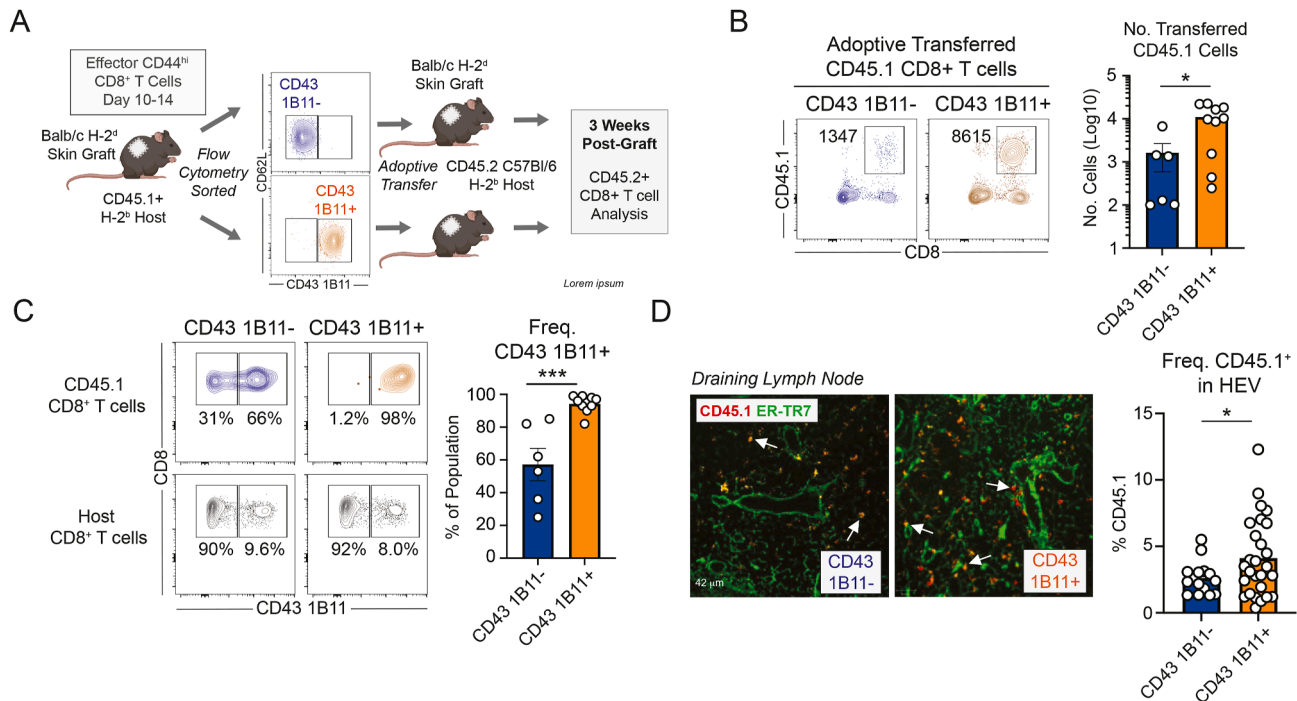


Figure 5. CD43 1B11⁺ CD8⁺ T cells are persistent after grafting relative to CD43 1B11⁻ CD8⁺ T cells. (A) Effector CD45.1 CD8⁺ T cells (postgraft days 10 to 14 after Balb/c skin grafts) were flow cytometrically sorted into CD43 1B11⁺ and CD43 1B11⁻ populations. Approximately 1×10^5 were adoptively transferred into C57Bl/6 (CD45.2) hosts, who were provided with Balb/c skin grafts the following day. Transferred cells were assessed on postgraft day 14. (B) Recovery of CD43 1B11⁺ and CD43 1B11⁻ CD8⁺ T cells in the secondary lymphoid organs. Numbers depict the absolute number of cells in the CD45.1⁺ gate. (C) Frequency of CD43 1B11⁺ CD8⁺ T cells among adoptively transferred populations. (D) Immunofluorescence histology of draining lymph nodes depicting adoptively transferred CD45.1 CD8⁺ T cells (red) and the HEV marker ER-TR7 (green). Arrows depict localization of adoptively transferred CD45.1 CD8⁺ T cells outside (CD43 1B11⁻) or within (CD43 1B11⁺) HEV. Summary data depicts pooled results from 3 independent experiments ($n = 6-10$ mice/group) and depicts mean $\pm$ SEM. Statistics performed by an unpaired Student's *t* test (2-tailed). Error bars depict SEM. HEV, high endothelial venule; SEM, standard error of the mean. * $P < .05$, ** $P < .01$, *** $P < .001$, **** $P < .0001$.

ICOS and CXCR3 (Fig. 7F). Overall, these data demonstrate that CD43 1D4 identifies a population of antigen-experienced CD8⁺ T cells in human healthy donors and renal transplant candidates.

3.10. CD43 1D4⁺ CD8⁺ T cells are efficient cytokine producers relative to CD43 1D4⁻ CD8⁺ T cells

To evaluate the functional capacity of CD43 1D4⁺ and CD43 1D4⁻ CD8⁺ T cells, we stimulated peripheral blood mononuclear cell from healthy donors with CD3 ϵ mAb for 18 to 20 hours and evaluated CD8⁺ T cells based on CD45RA and CD43 1D4 expression (Supplementary Fig. S4A and Fig. 7G). We found that CD3 ϵ mAb stimulation increased CD43 1D4 expression among both CD45RA^{hi} and CD45RA^{lo} populations (Supplementary Fig. S4B). This change was very modest among CD45RA^{lo} cells and was more pronounced among CD45RA^{hi} cells, presumably due to activation of a subset of naïve CD8⁺ T cells. We found that both CD45RA^{hi} and CD45RA^{lo} CD43 1D4⁺ fractions produced elevated levels of TNF- α and interferon γ relative to the CD43 1D4⁻ populations (Fig. 7H). In total, these data demonstrate that CD43 1D4 is a marker of antigen-experienced and acute effector CD8⁺ cells that are functional as determined by cytokine production.

4. Discussion

In this study, we built upon previous work that showed graft-specific L^d QL9⁺ CD8⁺ T cells express the activated glycoform of the sialomucin CD43, defined by the 1B11 mAb.¹⁹ We found that CD43 1B11 agonism with mAb was able to overcome subthreshold antigen/CD28 stimulation *in vitro* and break CD28/CD40L costimulation-blockade induced tolerance *in vivo*. These results suggest that CD43 1B11 signals may be redundant under robust activation conditions, but that it can provide a “rescue” stimulatory signal in the context of limited antigen and/or costimulation. This is potentially highly relevant for transplantation, as TCR signaling and costimulation pathways are targeted by maintenance immunosuppression.

On a population level, CD43 1B11⁺ status was highly sensitive for proliferating CD8⁺ T cells during acute rejection, and CD43 1B11⁺ CD8⁺ T cells were a significant fraction of graft-infiltrating effector CD8⁺ T cells during acute heart graft rejection. However, CD43 1B11⁺ CD8⁺ T cells also maintained expression of memory precursor and stem-like markers TCF-1 and IL-7R α , were more persistent after graft rechallenge, and were capable of forming memory cells. These results demonstrate that CD43 1B11⁺ CD8⁺ T cells are functionally distinct from a short-lived, terminally differentiated effector population. Future studies are needed to understand the programmatic

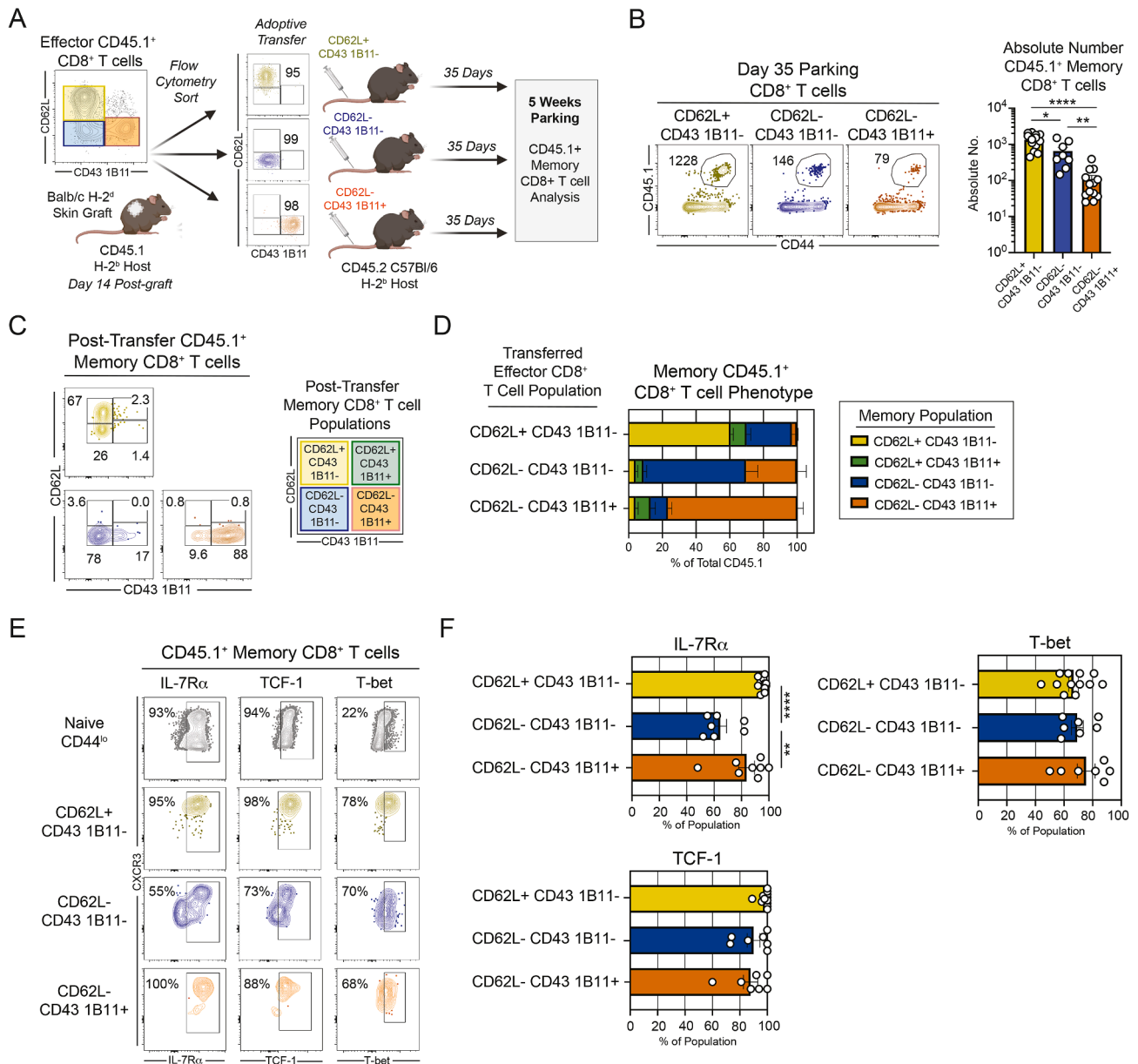


Figure 6. Effector CD43 1B11⁺ CD8⁺ T cells are maintained in memory in the absence of alloantigen. (A) Effector CD45.1 CD8⁺ T cells (postgraft days 12 to 14 after Balb/c skin grafts) were flow cytometrically sorted into CD62L^{hi}, CD43 1B11⁻, and CD43 1B11⁺ populations. Approximately 2×10^5 were adoptively transferred into naive C57Bl/6 (CD45.2) hosts and assessed on postgraft day 35 in the pooled draining lymphoid tissue and spleen. (B) Recovery of CD62L^{hi}, CD43 1B11⁻, and CD43 1B11⁺ adoptively transferred populations. Numbers depict the absolute number of cells in the CD45.1⁺ gate. (C) Representatively, flow CD62L and CD43 1B11 expression of transferred populations. (C) Representative flow plots and (D) summary data. (E) Representative flow plots and (F) summary data of IL-7R α , TCF-1, and T-bet expression among transferred populations. Summary data depicts pooled results from 3 independent experiments ($n = 8-13$ mice/group) and depicts mean $\pm$ SEM. Statistics performed by (B, E) 1-way ANOVA with Tukey's multiple comparisons. ANOVA, analysis of variance; IL-7R[α], Interleukin-7 receptor subunit α ; SEM, standard error of the mean; TCF-1, T cell factor 1. * $P < .05$, ** $P < .01$, *** $P < .0001$.

heterogeneity within CD43 1B11⁺ CD8⁺ T cells, including the defining transcription factors, whether CD43 1B11⁺ CD8⁺ T cells are predominantly self-renewing or continuously fed by CD43 1B11⁻ CD8⁺ T cells, and how memory CD43 1B11⁺ CD8⁺ T cells function upon rechallenge.

In humans, RNA-sequencing samples from patients with acute cellular rejection showed that effector SPN⁺GCNT1⁺ are present in rejecting allografts. Although samples within this data set without rejection did not include sufficient CD8⁺ T cells for

analysis (data not shown), these results provide evidence that CD8⁺ T cells expressing the activated CD43 receptor participate in acute rejection. Using an antibody for core-2 O-glycosylated human CD43, we showed that CD43 1D4⁺ defines a population of largely antigen-experienced effector CD8⁺ T cells. This marker does not strongly correlate with canonical CD45RA vs CCR7 effector/central memory populations. CD43 1D4⁺ CD8⁺ T cells were CD27^{lo}CXCR3^{hi}ICOS^{hi} and enriched for cytokine-producing cells, demonstrating that these are an activated

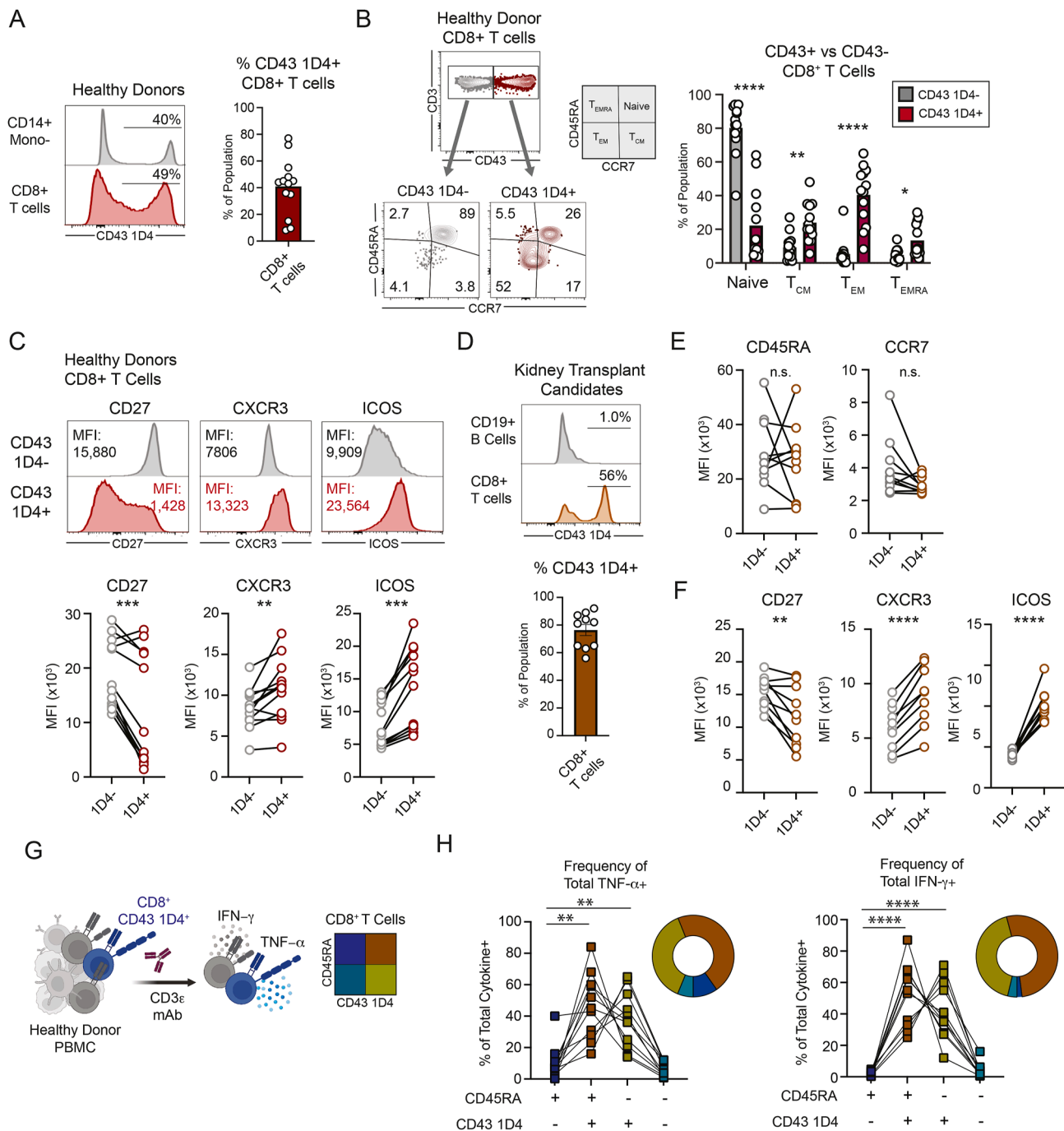


Figure 7. A population of human CD8⁺ T cells is CD43 1D4⁺ and has an antigen-experienced effector phenotype. (A) The frequency of peripheral healthy donor CD14⁺ monocytes and CD8⁺ T cells that express the CD43 epitope 1D4. (B, C) Expression of CD45RA and CCR7 among CD43 1D4⁺ and 1D4⁻ CD8⁺ T cells from healthy donors. (C) Representative flow plots and summary data showing expression of CD27, CXCR3, and ICOS among CD43 1D4⁺ and 1D4⁻ CD8⁺ T cell populations. (D) Frequency of CD43 1D4⁺ CD8⁺ T cells among kidney transplant candidates. (E) Expression of CD45RA, CCR7 among CD43 1D4⁺ and CD43 1D4⁻ CD8⁺ T cells from kidney transplant candidates. (F) Expression of CD27, CXCR3, and ICOS, among CD43 1D4⁺ and CD43 1D4⁻ CD8⁺ T cells from kidney transplant candidates. (G) Healthy donor PBMC were stimulated for 20 to 24 hours with CD3 ϵ mAb (OKT3) and assessed for TNF- α and interferon γ production. See also [Supplementary Figure S3](#). (H) Summary data depicting the proportion of TNF- α (left) or interferon γ (right) produced by each CD45RA and CD43 1D4 quadrant population. Each data point depicts an individual human donor. Summary data depict pooled results from (A-C) 3 independent experiments (n = 12-13 individuals/group), (D-F) 2 independent experiments (n = 10 individuals/group), and (H) 3 independent experiments (n = 11 individuals/group). Summary data depict mean $\pm$ SEM. Statistics performed by (B) 2-way ANOVA with Sidak's multiple comparisons test and (C, E) paired Student's *t* test (2-tailed) and (H) 1-way ANOVA with Dunnett's multiple comparisons test. Error bars depict SEM. ANOVA, analysis of variance; mAb, monoclonal antibody; PBMC, peripheral blood mononuclear cell; SEM, standard error of the mean; TNF, tumor necrosis factor. **P* < .05, ***P* < .01, ****P* < .001, *****P* < .0001.

effector T cell population. Further studies are needed to define the role of CD43⁺ CD8⁺ T cells in human allograft rejection, such as whether they correlate with the histologic grade of T cell-mediated rejection.

The main limitation of this work is the inability to genetically remove activated CD43 from CD8⁺ T cells during acute rejection in order to assess the role of CD43 1B11 in a reductionist system. Given the multiple defined roles of CD43 during T cell development, activation, and trafficking, it is likely that the removal of the entire CD43 receptor vs the activated glycoform of CD43 is a distinct biological question. In sum, this study provides a new understanding of the differentiation and fate of CD8⁺ T cells that mediate acute rejection beyond the canonical CD44^{hi} and CD62L^{lo} activation markers by defining activated CD43⁺ CD8⁺ T cells as highly activated alloantigen-specific effector CD8⁺ T cells in mice and humans.

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Author contributions

S.M.K., G.S.C., J.S.B., R.P.L., J.S.F., B.O., and D.C. conceptualized the study. G.S.C., J.S.F., M.W.S., R.H., Y.T., S.M.K., C.M., J.S.B., and R.W. performed experiments. S.M.K., G.S.C., S.B.B., and M.W.S. performed formal data analysis steps. S.M.K., G.S.C., J.S.F., and R.P.L. wrote the manuscript. S.M.K., J.S.B., C.M., and D.C. supervised the study personnel. All authors read and approved the final version of the paper.

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Declaration of competing interest

The authors of this manuscript have no conflicts of interest to disclose as described by *American Journal of Transplantation*.

Data availability

Further information and requests for resources and reagents should be directed to the lead contact, S.M.K. (skrumme1@jhmi.edu).

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ajt.2026.02.020>.

ORCID

Riley P. Leatham [ORCID](https://orcid.org/0009-0008-5688-2336) <https://orcid.org/0009-0008-5688-2336>
 Joel S. Freibaum [ORCID](https://orcid.org/0000-0002-1302-1279) <https://orcid.org/0000-0002-1302-1279>
 Gregory S. Cohen [ORCID](https://orcid.org/0000-0001-6652-4403) <https://orcid.org/0000-0001-6652-4403>
 Robin Welsh [ORCID](https://orcid.org/0000-0001-5822-0485) <https://orcid.org/0000-0001-5822-0485>
 Yukai Tang [ORCID](https://orcid.org/0009-0002-3423-6495) <https://orcid.org/0009-0002-3423-6495>
 Marina W. Shirkey [ORCID](https://orcid.org/0009-0005-7902-9045) <https://orcid.org/0009-0005-7902-9045>
 Sanniddhya B. Bardhan [ORCID](https://orcid.org/0009-0004-9915-8306) <https://orcid.org/0009-0004-9915-8306>
 Ryo Hatano [ORCID](https://orcid.org/0000-0002-2727-7275) <https://orcid.org/0000-0002-2727-7275>
 Chikao Morimoto [ORCID](https://orcid.org/0000-0002-3140-3596) <https://orcid.org/0000-0002-3140-3596>
 Byoung Chol Oh [ORCID](https://orcid.org/0000-0003-3337-7739) <https://orcid.org/0000-0003-3337-7739>
 Daniela Čiháková [ORCID](https://orcid.org/0000-0002-8713-2860) <https://orcid.org/0000-0002-8713-2860>
 Jonathan S. Bromberg [ORCID](https://orcid.org/0000-0002-1407-8795) <https://orcid.org/0000-0002-1407-8795>
 Scott M. Krummey [ORCID](https://orcid.org/0000-0002-7504-9162) <https://orcid.org/0000-0002-7504-9162>

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Hematopoietic dipeptidyl peptidase-4 deficiency promotes M2 macrophage polarization and attenuates atherosclerosis

Yuqi Zhou^{a,1}, Takeshi Matsumura^{a,b,*}, Sho Saito^a, Yoshitaka Yagi^{a,c}, Tomoaki Yoshinaga^a, Minoru Takeshita^{a,d}, Kazuki Fukuda^a, Takafumi Senokuchi^a, Hiroyuki Motoshima^c, Tatsuya Kondo^{a,e}, Ryo Hatano^f, Kei Ohnuma^f, Chikao Morimoto^f, Eiichi Araki^{d,g}, Naoto Kubota^a

^a Department of Metabolic Medicine, Faculty of Life Sciences, Kumamoto University, Kumamoto, Japan

^b Department of Medical Technology, Faculty of Health Sciences, Kumamoto Health Science University, Kumamoto, Japan

^c Kumamoto General Hospital, Kumamoto, Japan

^d Kikuchi Medical Association Hospital, Kikuchi, Japan

^e Aso Medical Center, Kumamoto, Japan

^f Department of Therapy Development and Innovation for Immune Disorders and Cancers, Graduate School of Medicine, Juntendo University, Tokyo, Japan

^g Research Center for Health and Sports Sciences, Kumamoto Health Science University, Kumamoto, Japan

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ABSTRACT

Atherosclerosis is a chronic inflammatory disease wherein macrophage polarization critically influences lesion development. Dipeptidyl peptidase-4 (DPP4), a serine protease expressed on immune cells, has been implicated in vascular inflammation; however, its cell type-specific roles remain unclear. This study aimed to determine whether *Dpp4* deficiency, particularly in hematopoietic cells, affects macrophage polarization and atherosclerosis progression. Using *Apoe*-knockout (*Apoe*KO) and *Apoe*- and *Dpp4*-double knockout mice as well as bone marrow transplantation models, we evaluated the impact of systemic and myeloid-specific *Dpp4* deficiency on macrophage phenotype and atherogenesis. In bone marrow-derived macrophages, *Dpp4* deficiency enhanced M2 marker expression (*Arg1*, *Ym1*, *Mgl2*, and *Fizz1*) and increased the proportion of CD206⁺ M2 macrophages without affecting M1 polarization, particularly after interleukin (IL)-4 stimulation. Furthermore, *in vivo* *Dpp4* deficiency significantly reduced aortic lesion area and macrophage infiltration in *Apoe*KO mice. Real-time reverse transcription-polymerase chain reaction analysis of aortic tissue revealed upregulation of M2-related genes and *Il4* expression, while M1-associated markers remained unchanged. Bone marrow transplantation experiments demonstrated that hematopoietic *Dpp4* deficiency was sufficient to suppress atherosclerosis, independent of systemic glucose or lipid alterations. This protective effect was accompanied by enhanced M2 polarization, suggesting a role for DPP4 in regulating macrophage phenotype. Collectively, these findings identify hematopoietic DPP4 as a key modulator of macrophage polarization and atherogenesis. *Dpp4* deficiency promotes the anti-inflammatory M2 phenotype and attenuates atherosclerosis regardless of metabolic effects, indicating that DPP4 may represent a promising therapeutic target for inflammatory cardiovascular diseases.

1. Introduction

Atherosclerosis is now recognized as a chronic inflammatory disease involving complex interactions between vascular cells, lipoproteins, and immune components [1,2]. Macrophages play pivotal roles throughout atherogenesis by contributing to lesion progression through the release

of pro-inflammatory cytokines, such as tumor necrosis factor- α (TNF- α) and interleukin-1 β (IL-1 β), and facilitating inflammation resolution via alternatively activated M2 macrophages [3–5]. Histological and experimental studies have demonstrated that M1 macrophages promote plaque formation and instability, whereas M2 macrophages facilitate repair and stabilization [6–8]. Therefore, enhancing M2 polarization is a

* Corresponding author. Department of Metabolic Medicine, Faculty of Life Sciences, Kumamoto University, 1-1-1 Honjo, Kumamoto, 860-5886, Japan.

E-mail address: takeshim@gpo.kumamoto-u.ac.jp (T. Matsumura).

¹ These authors contributed equally to this work.

promising strategy to limit atherosclerotic progression.

Macrophage polarization is regulated by multiple transcriptional pathways, including NF- κ B, STAT1/STAT6, and peroxisome proliferator-activated receptors (PPARs). Disruption of these signaling networks shifts macrophages toward a pro-inflammatory phenotype, accelerating lesion development. Conversely, promoting M2 differentiation suppresses inflammation and improves plaque stability, and identifying molecular regulators that control this balance remains an important therapeutic goal.

Dipeptidyl peptidase-4 (DPP-4), a serine protease best known for its role in glucose metabolism, is also expressed on immune cells, where it modulates inflammatory and immune responses [9,10]. Elevated circulating and tissue DPP4 levels have been associated with obesity and diabetes, both of which are closely linked to accelerated atherosclerosis. Beyond its enzymatic activity, DPP4 also functions as a signaling molecule via interaction with caveolin-1, influencing T-cell activation and cytokine release [11]. Pharmacological DPP4 inhibitors, widely used in diabetes treatment, have been shown to exert anti-inflammatory and anti-atherogenic effects independent of glycemic control [12].

We previously demonstrated that the DPP4 inhibitor, linagliptin, suppresses inflammation-induced DPP4 expression and increases M2 macrophages within atherosclerotic lesions [13], suggesting that DPP4 contributes to atherogenesis by regulating macrophage polarization. However, the direct impact of DPP4 deficiency in hematopoietic cells on macrophage phenotype and atherosclerosis remains unclear.

In this study, we employed whole-body *Dpp4*-knockout (*Dpp4*KO) and Apolipoprotein E-knockout (*Apoe*KO) double knockout (DKO) mice, as well as bone marrow transplantation models (*Dpp4*KO $\rightarrow$ *Apoe*KO), to determine whether the absence of DPP4, particularly in hematopoietic-derived cells, enhances M2 macrophage polarization and suppresses atherosclerotic lesion formation.

2. Materials and methods

2.1. Materials

Lipopolysaccharide (LPS) from *Escherichia coli* O111:B4 was purchased from Wako Chemicals (Tokyo, Japan). Recombinant murine interferon- γ (IFN- γ ; 315-05) was purchased from PeproTech, Inc. (Rocky Hill, NJ). Interleukin-4 (IL-4) was generously provided by FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). Rat monoclonal antibodies were purchased as follows: PE-Cy7-conjugated anti-F4/80 (catalog number 123114), Alexa Fluor488-conjugated anti-CD86 (catalog number 105018), and PE-conjugated anti-CD206 (catalog number 141706) from BioLegend (San Diego, CA); PerCP-Cy5.5-conjugated anti-CD11b (catalog number 561114) from BD Biosciences (Franklin Lakes, NJ); and Alexa Fluor488-conjugated anti-iNOS (NOS2) (catalog number 53-5920-80) from eBioscience, Inc. (San Diego, CA). All other chemicals were of the highest grade available from commercial sources.

2.2. Animals

*Apoe*KO mice (C57BL/6 background) were obtained from Jackson Laboratory, and *Dpp4*KO mice were kindly provided by Dr. Chikao Morimoto (Juntendo University, Tokyo, Japan). DKO mice were generated by crossbreeding. Mice were housed under controlled conditions (22 °C, 12 h light/dark cycle) with free access to standard chow and water. All animal procedures conformed to institutional guidelines and were approved by the Animal Ethics Committee of Kumamoto University (A2024-0812).

At 12 weeks of age, mice were fed either a normal chow diet for 8 weeks. Glucose and insulin tolerance tests were performed as previously described [14]. Mice were euthanized under isoflurane anesthesia, and aortas and serum were collected for histological and biochemical analyses. Lipid profiles (total cholesterol, triglyceride, high-density lipoprotein-cholesterol, low-density lipoprotein-cholesterol, chylomicron-

cholesterol, and very low-density lipoprotein-cholesterol), were measured at Skylight Biotech Inc. (Akita, Japan). Aortic lesions were visualized by Oil Red O staining, and lesion areas were quantified using BZ-X700 microscopy software (Keyence, Tokyo, Japan) [14].

2.3. Immunohistochemistry

Frozen aortic sinus sections (6 μ m) were incubated with primary antibodies overnight at 4 °C, followed by fluorescent secondary antibodies. Nuclei were counterstained with DAPI (4',6-diamidino-2-phenylindole) solution [13]. Fluorescent images were obtained using a BZ-X700 microscope (Keyence), and lesion areas were quantified using Image analysis software [13].

2.4. Bone marrow-derived macrophages (BMMs)

Bone marrow cells were isolated from the femurs and tibias of 6–8-week-old male *Apoe*KO or DKO mice and cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10 % fetal bovine serum, antibiotics, and 20 % L-cell-conditioned medium as a source of macrophage colony-stimulating factor. After 7 days, adherent cells were collected as mature BMMs [15].

2.5. Flow cytometry

For *in vivo* analysis, aortas were digested with collagenase I/XI, DNase I, and hyaluronidase at 37 °C for 1 h [13]. Cell suspensions were stained with antibodies against CD11b, F4/80, CD86 (M1 marker), and CD206 (M2 marker).

For *in vitro* analysis, BMMs were detached using 5 mM ethylenediaminetetraacetic acid, washed, and stained with antibodies against iNOS and CD206. Data were acquired using an SH800 cell sorter (Sony, Tokyo, Japan) and analyzed with FlowJo v10 software (Tree Star, Inc., Portland, OR) [13].

2.6. Quantitative Reverse Transcription Polymerase Chain Reaction (RT-PCR)

Total RNA from BMMs or aortas was isolated with the RNeasy Mini Kit (Qiagen, Hilden, Germany) and reverse-transcribed using ReverTra Ace qPCR RT Master Mix (Toyobo, Osaka, Japan) [13]. Gene expression was quantified by real-time PCR using SYBR Green and normalized to 18S rRNA. Primer sequences are provided in Supplemental Table 1.

2.7. Bone marrow transplantation

Bone marrow cells were harvested from the femurs and tibias of donor mice (*Apoe*KO or *Dpp4*/*Apoe* DKO) and suspended in sterile DMEM on ice. Recipient mice (*Apoe*KO or DKO, 6–8 weeks old) were lethally irradiated with a single 9.5 Gy dose of total body irradiation and reconstituted with donor bone marrow cells (1×10^7 cells) via tail vein injection. Four experimental groups were generated: (1) *Apoe*KO $\rightarrow$ *Apoe*KO (control), in which both hematopoietic and non-hematopoietic compartments were *Apoe* deficient; (2) DKO $\rightarrow$ *Apoe*KO (hematopoietic *Dpp4* deficiency); (3) *Apoe*KO $\rightarrow$ DKO (non-hematopoietic *Dpp4* deficiency); and (4) DKO $\rightarrow$ DKO (systemic *Dpp4*/*Apoe* deficiency). This design allowed us to distinguish the specific contribution of hematopoietic versus non-hematopoietic *Dpp4* deficiency to atherosclerosis progression. Successful engraftment and donor cell reconstitution were confirmed by genotyping peripheral blood samples collected four weeks after transplantation [16].

2.8. Statistical analysis

All data are expressed as mean $\pm$ SEM. Differences between the two groups were evaluated by an unpaired *t*-test with Welch's correction,

and multiple comparisons were analyzed by one-way ANOVA followed by Tukey's post hoc test. $P < 0.05$ was considered statistically significant.

3. Results

3.1. *Dpp4* deficiency affected M2 macrophage polarization in BMMs

First, we investigated the effect of *Dpp4* deficiency on macrophage differentiation under various stimuli. Following inflammatory stimulation with LPS and IFN- γ , the expression of key M1 macrophage marker genes, including *Il1b*, *Mcp1*, *Tnfa*, and *Nos2*, was markedly upregulated. However, *Dpp4* knockout had no significant effect on this upregulation (Fig. 1A). Conversely, while LPS/IFN- γ stimulation markedly suppressed the expression of M2 macrophage markers such as *Mgl2* and *Ym1*, *Dpp4*

deficiency partially restored their expression under these inflammatory conditions (Fig. 1B). Consistent with previous reports, IL-4 stimulation had no significant effect on the expression of M1 macrophage markers (Fig. 1C). In contrast, the spontaneous upregulation of M2 macrophage markers observed in *Dpp4*-deficient cells was further augmented by IL-4 stimulation (Fig. 1D). In recent years, M2 macrophages have been further subclassified into distinct phenotypes. Among these, those induced by IL-4 stimulation are specifically referred to as M2a macrophages. Our real-time RT-PCR analysis showed that IL-4 stimulation upregulated M2a macrophage-specific markers (*Fizz1* and *Il1ra*), and that *Dpp4* deficiency further and significantly enhanced this upregulation (Fig. 1E).

Flow cytometric analysis corroborated findings from previous experiments. Within the macrophage population defined as F4/80⁺CD11b⁺ cells, M1 differentiation was induced by inflammatory

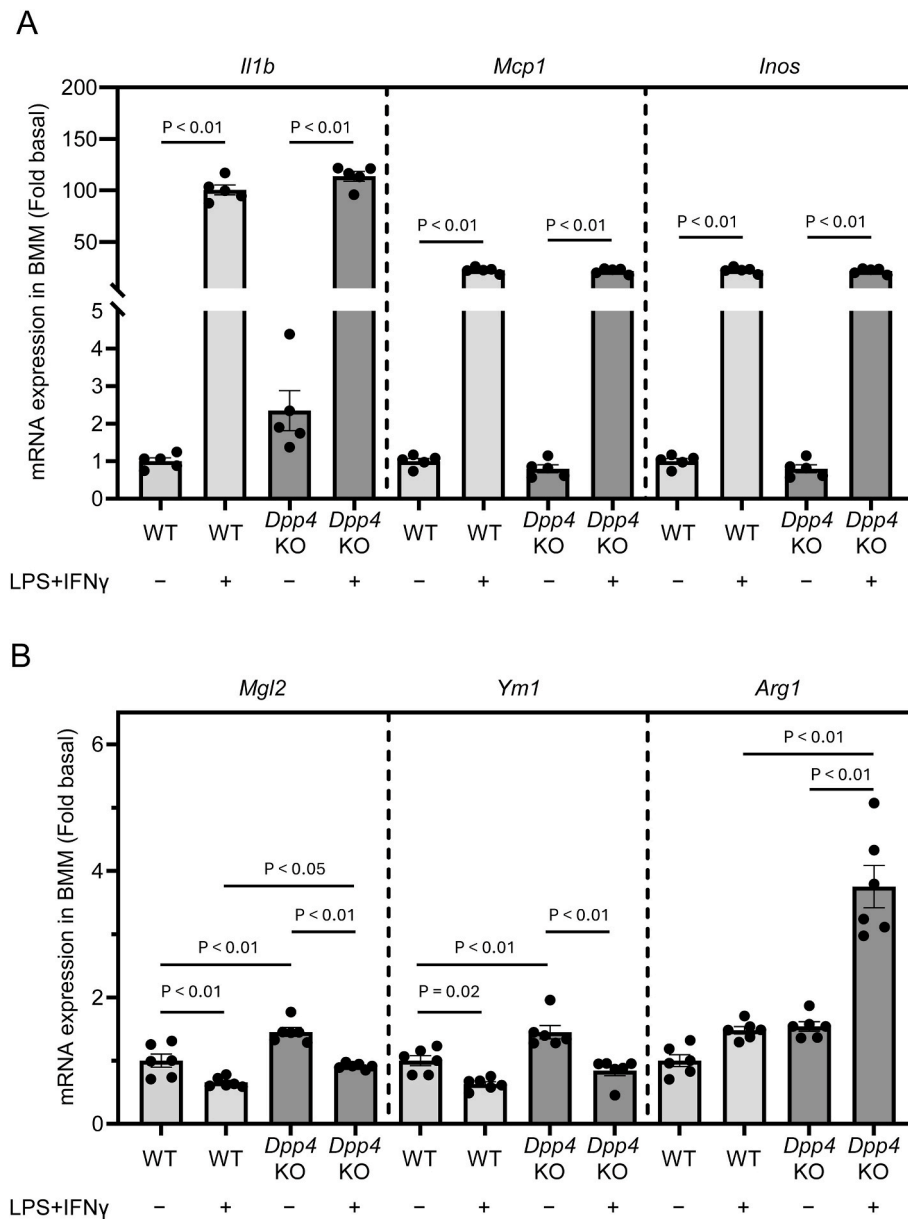


Fig. 1. *Dpp4* deficiency promotes M2 macrophage polarization in bone marrow-derived macrophages (BMMs)

(A–B) Real-time RT-PCR analysis of M1 macrophage markers (*Il1b*, *Mcp1*, *Nos2*) (A) and M2 markers (*Mgl2*, *Ym1*, *Arg1*) (B) in BMMs stimulated with LPS and IFN- γ . (C, D) Real-time RT-PCR analysis of M1 macrophage markers (*Il1b*, *Mcp1*, *Nos2*) (C), M2 markers (*Mgl2*, *Ym1*, *Arg1*) (D), and M2a-specific markers (*Il1ra*, *Fizz1*) (E) in BMMs stimulated with IL-4. (F–I) Flow cytometry showing proportions of CD86⁺ (M1) and CD206⁺ (M2) macrophages among F4/80⁺CD11b⁺ cells in BMMs stimulated with LPS and IFN- γ (F, G), or IL-4 (H, I). Data represent mean $\pm$ SEM; $P < 0.05$

BMM, bone marrow-derived macrophages; LPS, lipopolysaccharide; RT-PCR, reverse transcription polymerase chain reaction.

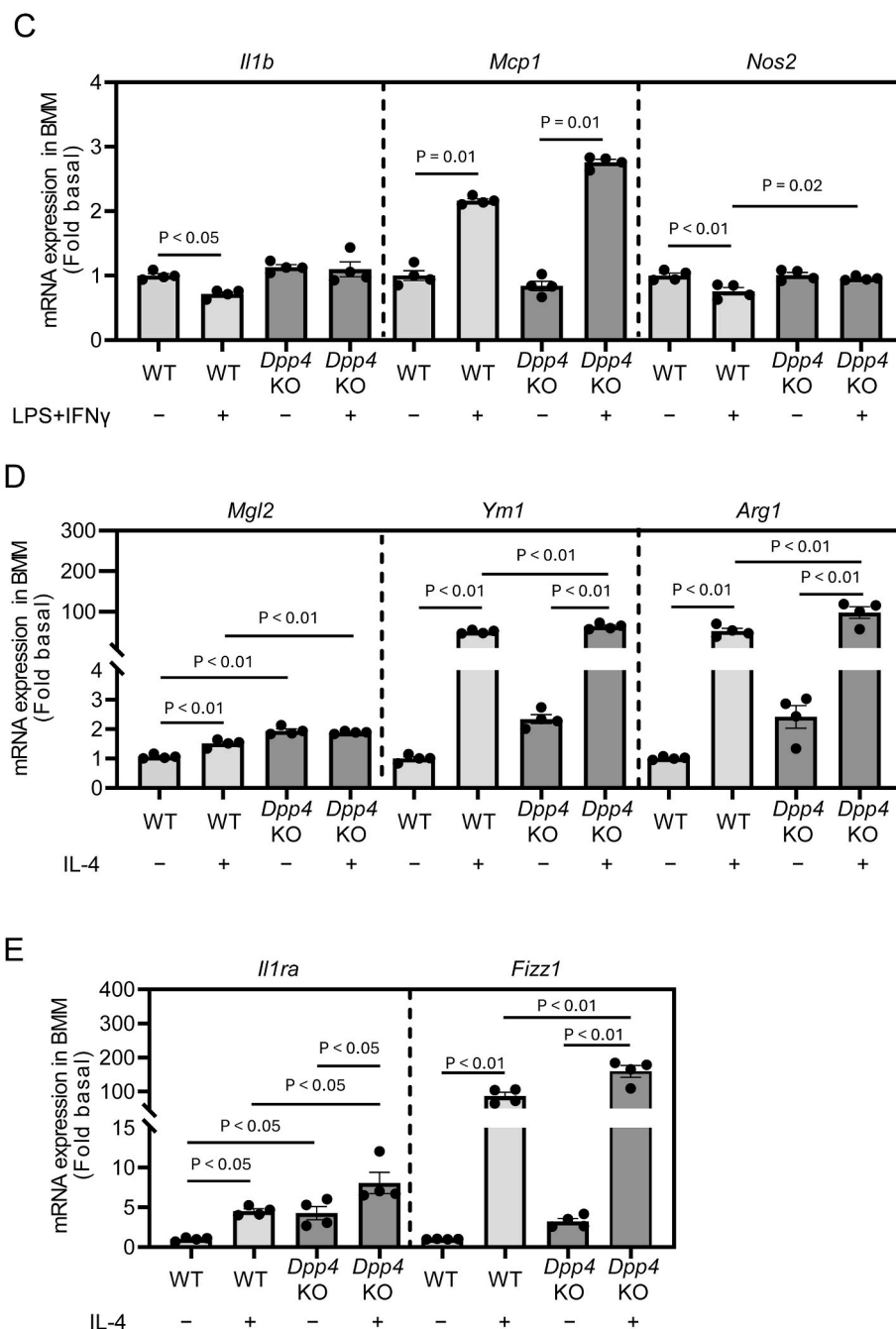


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stimuli but remained unaltered by IL-4 stimulation. In contrast, the proportion of M2 macrophages was reduced by inflammatory stimulation, and this reduction was significantly rescued in *Dpp4*-deficient cells. Moreover, *Dpp4* deficiency intrinsically promoted M2 polarization, an effect that was further augmented by IL-4 stimulation (Fig. 1F–I).

3.2. *Dpp4* deficiency attenuates atherosclerosis progression and inhibits macrophage infiltration into atherosclerotic plaques in *Apoe*KO mice

Based on the significant *in vitro* findings, we subsequently performed an *in vivo* study to examine how *Dpp4* deficiency influences atherosclerotic lesion formation. Both groups were monitored weekly throughout the study, and no significant differences were observed in body weight (Supplemental Fig. 1A). The DKO group showed improved glucose metabolism, with significantly lower casual blood glucose levels at

multiple time points (Supplemental Fig. 1B) and enhanced glucose tolerance in the intraperitoneal glucose tolerance test compared with controls (Supplemental Fig. 1C). However, the insulin tolerance test revealed no significant difference in insulin sensitivity between the two groups (Supplemental Fig. 1D). Serum lipid levels were also comparable between groups (Supplemental Fig. 1E). At the end of the study, liver weight was similar between groups, whereas visceral fat mass was significantly reduced in the DKO group (Supplemental Fig. 1F and G). Histological analysis of the aortic sinus and thoracic aorta stained with Oil Red O clearly demonstrated that *Dpp4* deficiency suppressed atherosclerotic lesion development in normal chow-fed *Apoe*KO mice (Fig. 2A and B).

Immunofluorescence staining of heart sections revealed that macrophage infiltration (F4/80⁺ cells) within atherosclerotic plaques was significantly reduced in DKO mice (Fig. 2C). Consistently,

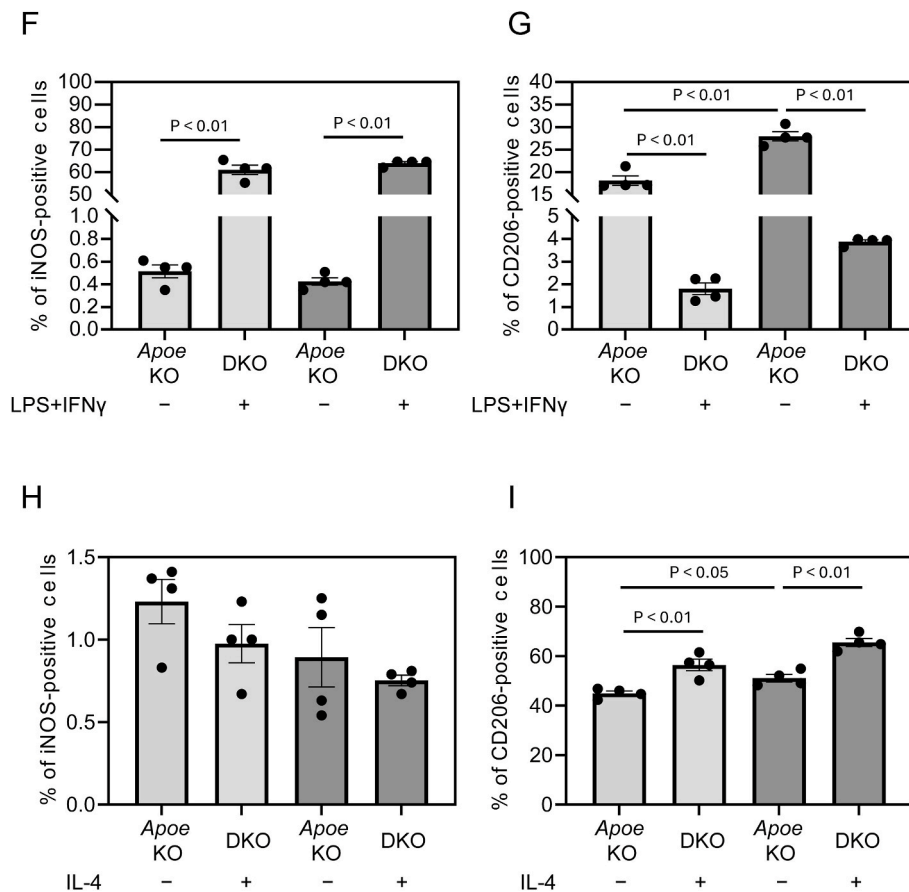


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quantitative analysis of mRNA extracted from whole aortic plaques showed a marked decrease in F4/80 mRNA expression in the DKO group, further supporting the reduced macrophage infiltration observed histologically (Fig. 2D).

3.3. *Dpp4* deficiency promotes M2 macrophage polarization in the aorta

Real-time RT-PCR analysis of the aortas revealed that *Dpp4* deficiency did not significantly alter the mRNA expression levels of M1 macrophage markers (Fig. 3A). In contrast, *Dpp4* deficiency markedly increased the expression of several M2 macrophage markers (Fig. 3B), including the canonical markers *Mgl2*, *Ym1*, and *Arg1* as well as M2a subtype-specific markers such as *Ccl17*, *Il1ra*, and *Fizz1*.

Flow cytometric analysis demonstrated that *Dpp4* deficiency did not affect the number of M1 macrophages (CD86⁺ cells) in the aorta (Fig. 3C) but significantly increased the number of M2 macrophages (CD206⁺ cells) (Fig. 3D).

Furthermore, RT-PCR analysis of aortic mRNA revealed that *Dpp4* deficiency significantly increased *Il4* expression (Fig. 3E), consistent with the marked upregulation of multiple M2 macrophage markers observed in *Dpp4*KO mice (Fig. 3B).

3.4. Impact of myeloid-specific *Dpp4* deficiency on atherosclerosis progression evaluated by bone marrow transplantation

To exclude the potential influence of *Dpp4* deficiency on glucose metabolism, we conducted experiments using a bone marrow transplantation model. We found no significant differences among the four experimental groups in body weight (Supplemental Fig. 2A), casual blood glucose levels (Supplemental Fig. 2B), glucose tolerance tests (Supplemental Fig. 2C), insulin tolerance tests (Supplemental Fig. 2D),

or serum lipid profiles (Supplemental Fig. 2E). All three experimental groups (DKO→DKO, DKO→ApoeKO, and ApoeKO→DKO) showed a significant reduction in atherosclerotic lesion area compared with the ApoeKO→ApoeKO control group (Fig. 4A). The most pronounced anti-atherogenic effect was observed in the DKO→DKO group, while no significant difference was detected between the DKO→ApoeKO and ApoeKO→DKO groups.

Real-time RT-PCR analysis showed no significant differences in the mRNA expression levels of M1 macrophage markers among the four groups (Fig. 4B–E). In contrast, *Dpp4* deficiency significantly increased the expression of M2 macrophage markers (Fig. 4F–H–J), with *Ym1* showing a similar trend (Fig. 4G). Notably, the absence of *Dpp4* in bone marrow-derived cells was sufficient to enhance the expression of M2 markers such as *Mgl2* and *Fizz1*; this effect was markedly stronger than that observed with *Dpp4* deficiency in non-hematopoietic tissues.

4. Discussion

This study demonstrates that *Dpp4* deficiency markedly attenuates atherosclerotic lesion development in mice, primarily through myeloid-specific deletion that enhances M2 macrophage polarization. These findings extend our previous observation that pharmacological DPP4 inhibition increases M2 macrophages within atherosclerotic plaques [13]. Moreover, hematopoietic DPP4 is identified as a key regulator linking immune modulation and vascular inflammation.

Importantly, our genetic models allowed us to exclude glycemic effects, as *Dpp4*-deficient mice showed no significant changes in serum lipids or glucose metabolism. Thus, the atheroprotective effects observed here likely reflect a glucose-independent mechanism. This aligns with a recent study wherein DPP4 inhibition improved plaque stability via non-metabolic pathways, such as suppression of senescence-

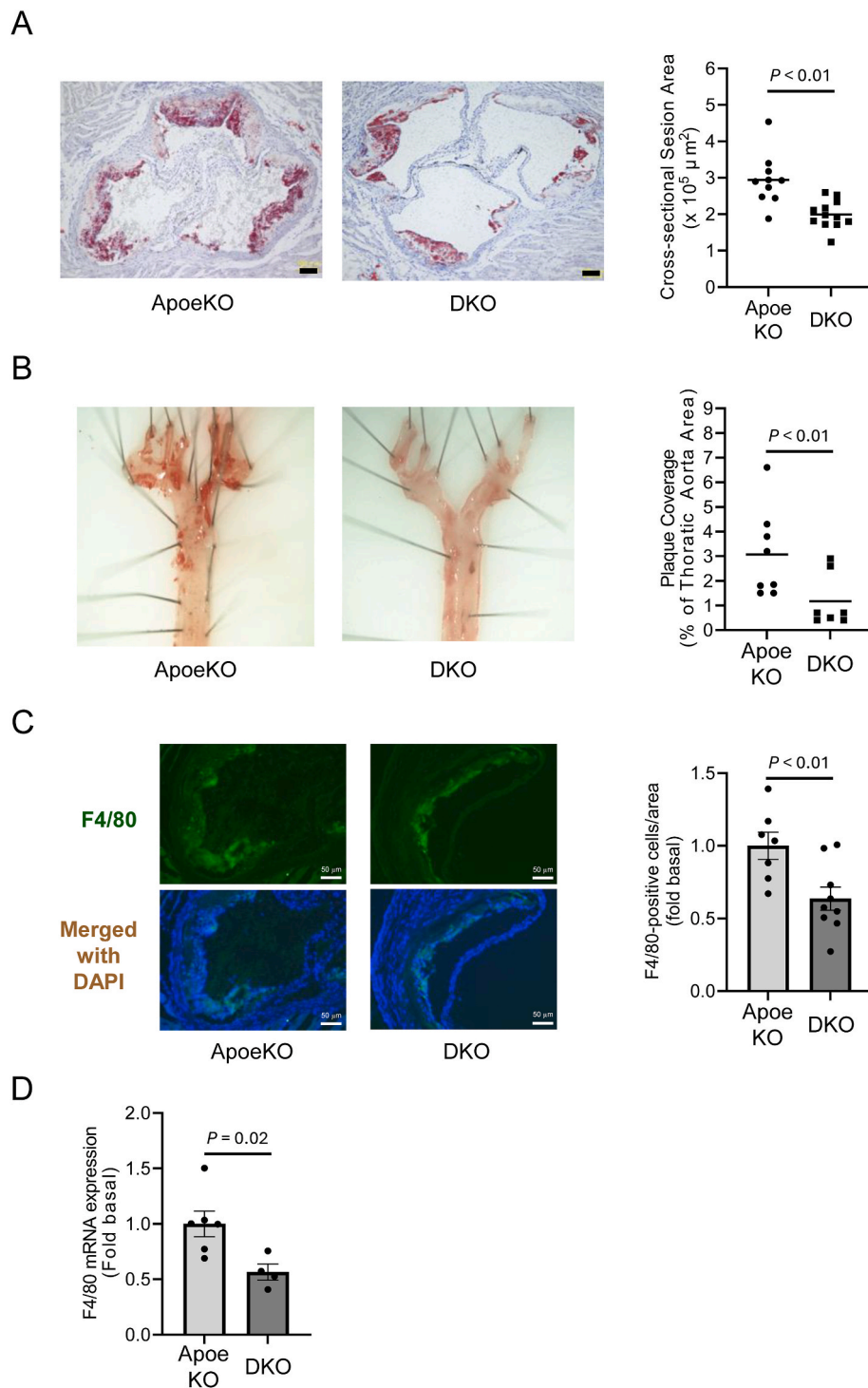


Fig. 2. *Dpp4* deficiency suppresses atherosclerosis and macrophage infiltration in ApoeKO mice

(A, B) Representative Oil Red O-stained aortic sinus and thoracic aorta sections from ApoeKO and *Dpp4*/Apoe double knockout (DKO) mice. (C) Immunofluorescence staining for F4/80⁺ macrophages in aortic plaques. (D) Quantitative RT-PCR of aortic F4/80 mRNA confirming reduced macrophage infiltration. Data represent mean $\pm$ SEM; $P < 0.05$. RT-PCR, reverse transcription polymerase chain reaction. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

associated hemostasis [17]. Our findings further support the concept that DPP4 in myeloid cells directly modulates inflammatory signaling rather than merely influencing metabolic control.

Consistent with a recent report demonstrating that hematopoietic *Dpp4* deficiency reduces atherosclerosis and T-cell-mediated vascular inflammation in *Ldlr*^{-/-} mice [18], our bone marrow transplantation experiments revealed that loss of hematopoietic *Dpp4* is sufficient to

suppress atherogenesis. Although their study focused on T-cell migration, we identified M2 macrophage polarization as a complementary mechanism contributing to the same protective phenotype. These results suggest that hematopoietic DPP4 promotes atherosclerosis through multiple immune pathways.

Our bone marrow transplantation model also distinguished systemic *Dpp4*/Apoe deficiency from the hematopoietic effects of *Dpp4* deficiency.

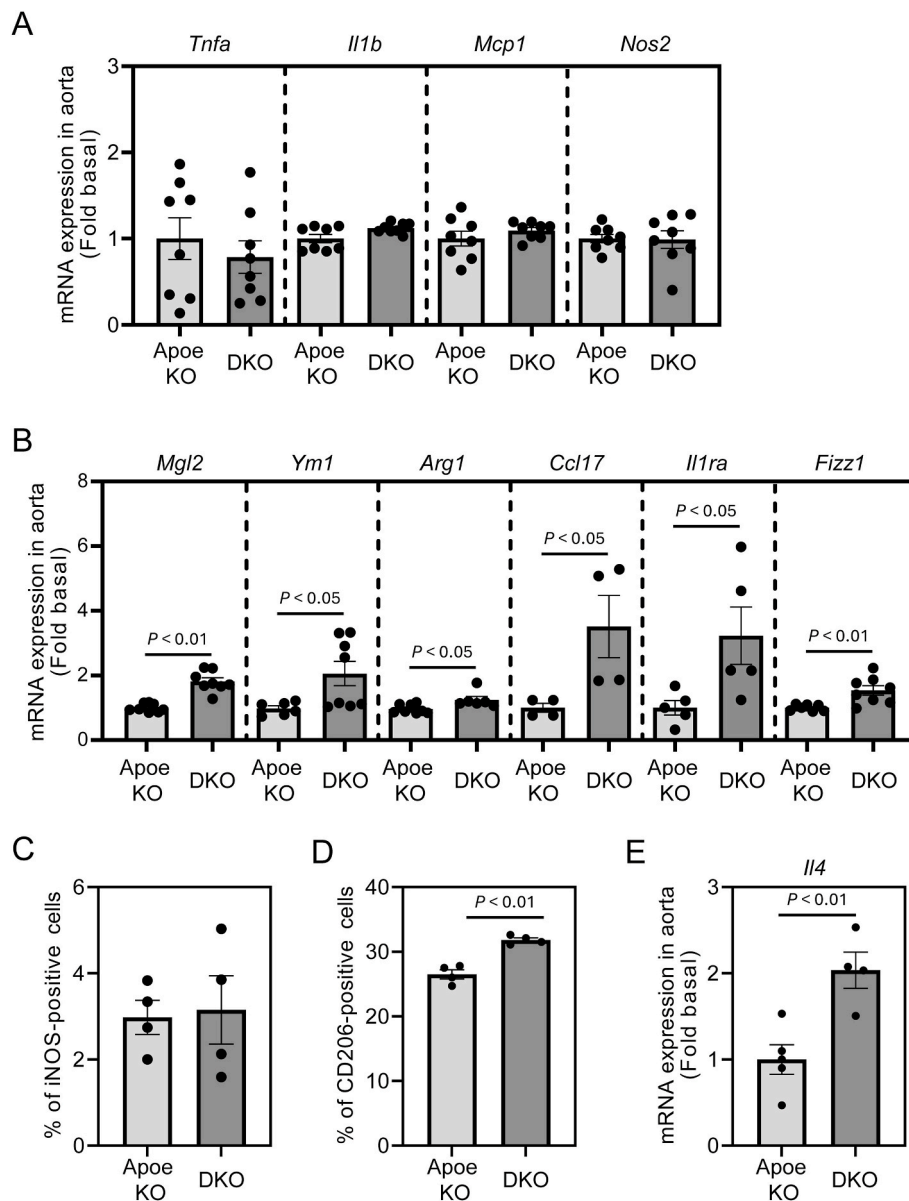


Fig. 3. *Dpp4* deficiency enhances M2 macrophage polarization in the aorta

(A) The mRNA expression levels of M1 markers (*Tnfa*, *Il1b*, *Mcp1*, *Nos2*) in the aorta were determined by real-time RT-PCR. (B) The mRNA expression levels of M2 markers (*Mgl2*, *Ym1*, *Arg1*) and M2a markers (*Ccl17*, *Il1ra*, *Fizz1*) in the aorta were determined by real-time RT-PCR. (C, D) Flow cytometry showing proportions of iNOS⁺ (M1) (C) and CD206⁺ (M2) (D) macrophages among F4/80⁺CD11b⁺ cells in the aorta of ApoeKO or DKO mice. (E) The mRNA expression levels of *Il4* in the aorta were determined by real-time RT-PCR. Data represent mean $\pm$ SEM; *P* < 0.05. DKO, double knockout; RT-PCR, reverse transcription polymerase chain reaction.

All transplanted groups showed comparable metabolic parameters, excluding confounding factors such as insulin resistance or adiposity. Although total-body irradiation used for preconditioning may itself promote vascular injury [19], *Dpp4*-deficient chimeras still exhibited significant suppression of atherosclerosis, indicating a robust protective effect.

Mechanistically, our results implicate enhanced M2 macrophage polarization in mediating this atheroprotection. The absence of *Dpp4* may potentiate IL-4/STAT6 signaling, a central pathway for M2 differentiation. STAT6 activation stabilizes plaques by suppressing oxidized LDL-induced apoptosis and inflammation [20], and its activity is further regulated through interaction with TRIM24 [21]. It is plausible that *Dpp4* deficiency augments this signaling cascade, thereby promoting M2 polarization and reducing lesion progression.

Nuclear receptors of the PPAR family also govern macrophage

polarization and exert anti-atherogenic effects [22]. Endogenous and pharmacological PPAR α and PPAR δ ligands promote M2-like phenotypes and reduce vascular inflammation [23,24]. *Dpp4* deficiency may indirectly activate one or more of these pathways, reinforcing the anti-inflammatory macrophage profile observed in this study.

Despite offering these insights, this study has several limitations. First, we did not employ DPP4 overexpression or agonist models, which would be necessary to confirm the causal relationship between DPP4 activity and atherosclerosis progression. Moreover, elevated circulating DPP4 levels have been associated with hyperinsulinemia and endothelial dysfunction [25], suggesting that DPP4 exerts both enzymatic and signaling effects on vascular homeostasis. DPP4 expression on monocytes increases with obesity and dyslipidemia [10,26], linking metabolic inflammation to cardiovascular disease. Mechanistically, DPP4 interacts with caveolin-1 in antigen-presenting cells, triggering NF- κ B-dependent

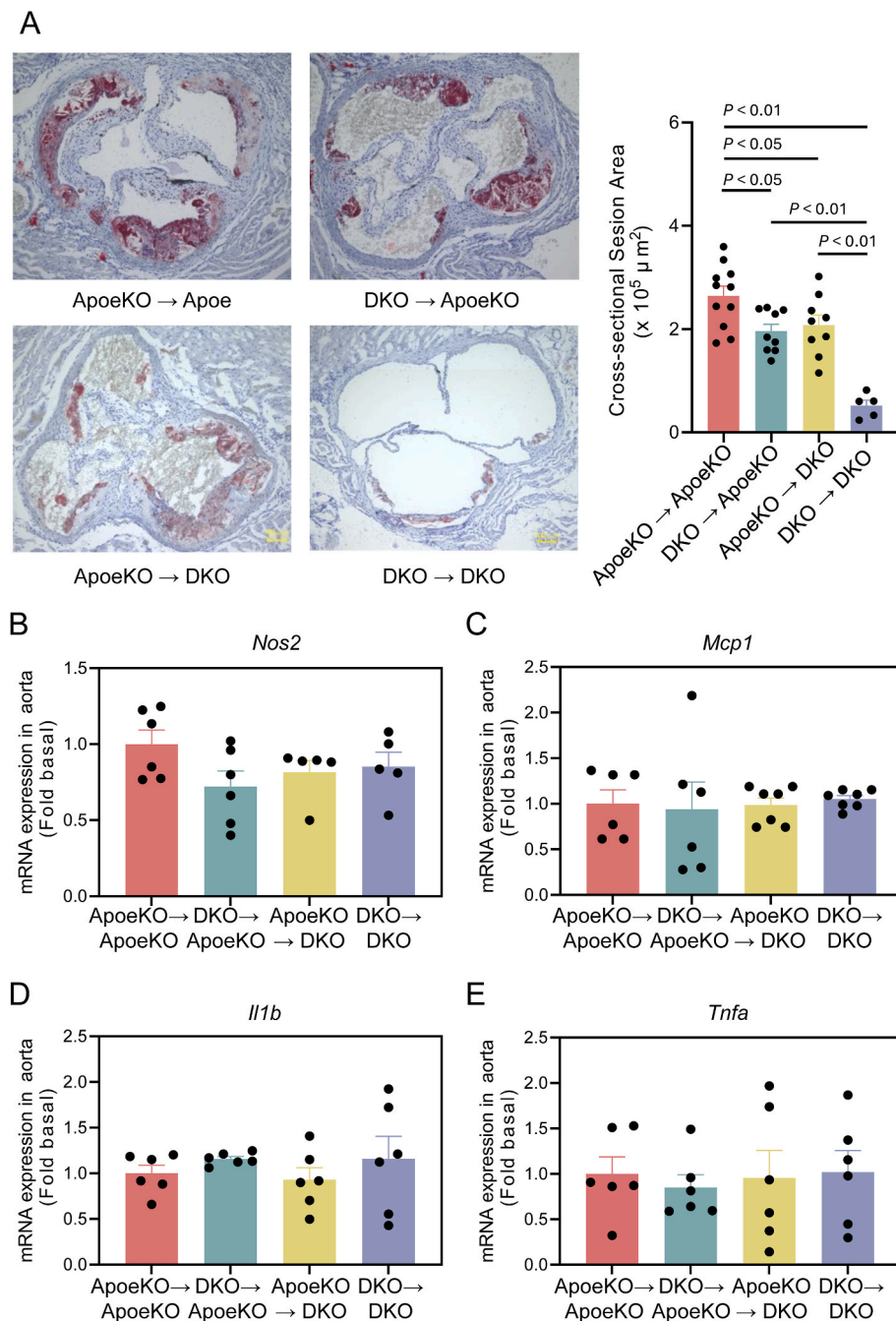


Fig. 4. Myeloid *Dpp4* deficiency attenuates atherosclerosis in bone marrow transplantation (BMT) models

(A) Aortic lesion area in four BMT groups: ApoeKO → ApoeKO (control), DKO → ApoeKO, ApoeKO → DKO, and DKO → DKO. (B–E) The mRNA expression levels of M1 markers (*Nos2*, *Mcp1*, *Il1b*, *Tnfa*) in the aorta were determined by real-time RT-PCR. (F–J) The mRNA expression levels of M2 markers (*Mgl2*, *Ym1*, *Arg1*) and M2a markers (*Ccl17*, *Il1ra*, *Fizz1*) in the aorta were determined by real-time RT-PCR. Data represent mean $\pm$ SEM; $P < 0.05$. RT-PCR, reverse transcription polymerase chain reaction.

T-cell activation [27]; it also regulates inflammatory peptide activity in rheumatoid arthritis [28]. These dual enzymatic and non-enzymatic functions likely underlie its broader pro-inflammatory role.

Finally, our work focused on the IL-4-induced M2a subtype; however, macrophages represent a spectrum of phenotypes, including M2b and M2c subtypes that are induced by immune complexes or IL-10/TGF- β , respectively [29,30]. Recent studies have described metabolically activated macrophages (MMe, Mox) that drive obesity-induced inflammation and insulin resistance [31,32]. However, it is unclear whether DPP4 influences these non-classical subsets. Future studies should

determine how DPP4 deficiency affects distinct macrophage programs to provide a comprehensive view of its immunometabolic functions.

Overall, our findings identify hematopoietic DPP4 as a key regulator of macrophage polarization and atherogenesis. The genetic deletion of DPP4 enhances M2 macrophage differentiation and attenuates lesion development regardless of metabolic effects, suggesting that DPP4 is a promising therapeutic target for inflammatory cardiovascular diseases.

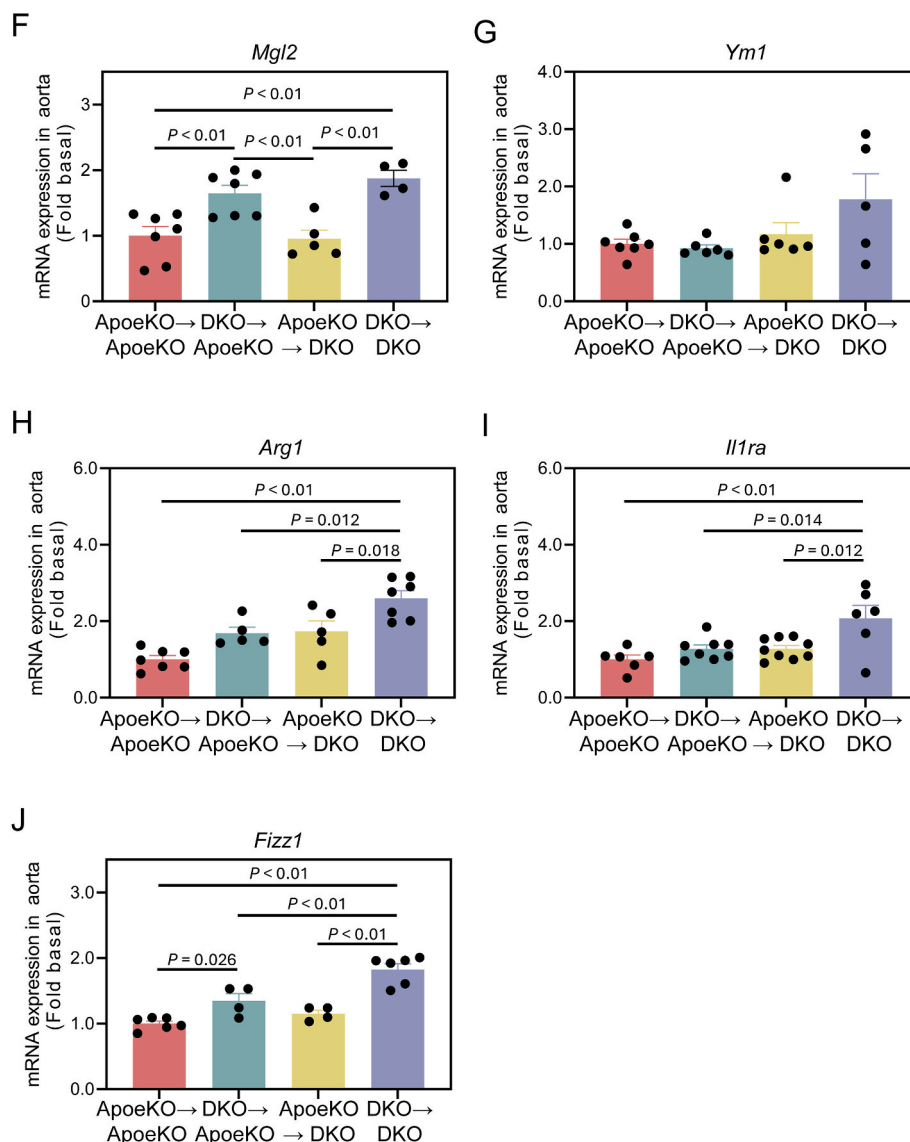


Fig. 4. (continued).

CRedit authorship contribution statement

Yuqi Zhou: Formal analysis, Investigation, Methodology, Writing – original draft. **Takeshi Matsumura:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Methodology, Writing – original draft, Writing – review & editing. **Sho Saito:** Formal analysis, Investigation. **Yoshitaka Yagi:** Formal analysis, Investigation. **Tomoaki Yoshinaga:** Formal analysis, Investigation. **Minoru Takeshita:** Formal analysis, Investigation. **Kazuki Fukuda:** Formal analysis, Investigation. **Takafumi Senokuchi:** Formal analysis, Investigation. **Hiroyuki Motoshima:** Formal analysis, Investigation. **Tatsuya Kondo:** Formal analysis, Investigation. **Ryo Hatano:** Conceptualization, Resources. **Kei Ohnuma:** Resources. **Chikao Morimoto:** Resources. **Eiichi Araki:** Supervision, Writing – review & editing. **Naoto Kubota:** Supervision, Writing – original draft, Writing – review & editing.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bbrc.2025.153037>.

Data availability

Data supporting the findings are available from the corresponding author upon reasonable request.

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6 Five-Year Clinical Outcomes With Nivolumab Plus Ipilimumab Versus Chemotherapy as First-Line Treatment for Unresectable Pleural Mesothelioma in CheckMate 743

Arnaud Scherpereel, MD, PhD¹ ; Paul Baas, MD, PhD² ; Anna K. Nowak, MBBS, PhD³ ; Anne S. Tsao, MD, MBA⁴ ; Nobukazu Fujimoto, MD, PhD⁵; Solange Peters, MD, PhD⁶ ; Aaron S. Mansfield, MD⁷ ; Sanjay Popat, FRCP, PhD⁸ ; Yolanda Bautista Aragon, MD⁹; Toby Talbot, MD¹⁰; Francesco Grossi, MD¹¹ ; Dariusz Kowalski, MD, PhD¹² ; Muhammet A. Kaplan, MD¹³; Andrés Felipe Cardona, MD, PhD^{14,15} ; Raheela Soomro, MD¹⁶; Nan Hu, MS¹⁶; Adam Lee, MSc¹⁷ ; Virginia Ip, PhD¹⁶; Yu-Han Hung, PhD¹⁶ ; and Gérard Zalcman, MD, PhD¹⁸ 

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ABSTRACT

In this 5-year follow-up from the CheckMate 743 study in patients with unresectable pleural mesothelioma (PM), we evaluated updated efficacy and safety outcomes and biomarkers and performed treatment-switching analyses with first-line nivolumab plus ipilimumab versus chemotherapy. With a median follow-up of 66.8 months, nivolumab plus ipilimumab demonstrated continued overall survival (OS) benefit versus chemotherapy in all randomly assigned patients (5-year OS rates, 14% v 6%; hazard ratio [HR], 0.74 [95% CI, 0.62 to 0.88]); similar benefit was observed regardless of tumor histology. Of biomarker-evaluable patients treated with nivolumab plus ipilimumab ($n = 242$), high baseline monocytic myeloid-derived suppressor cell (M-MDSC) levels correlated with worse OS versus low M-MDSC levels (HR, 1.25 [95% CI, 1.09 to 1.43]). After adjusting for 24% of patients in the chemotherapy arm who received subsequent immunotherapy, nivolumab plus ipilimumab demonstrated continued OS benefit versus chemotherapy (HR, 0.64 [95% CI, 0.53 to 0.78]). No new safety signals were observed. These results demonstrate long-term, durable clinical benefit with nivolumab plus ipilimumab versus chemotherapy, which continued to be preserved even after treatment-switching adjustment in the chemotherapy arm. Exploratory analyses suggested greater benefit with nivolumab plus ipilimumab in the low M-MDSC subgroup. These results further support first-line nivolumab plus ipilimumab as standard of care for unresectable PM.

ACCOMPANYING CONTENT

-  [Data Sharing Statement](#)
-  [Data Supplement](#)
-  [Protocol](#)

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INTRODUCTION

Nivolumab plus ipilimumab has demonstrated long-term, durable survival benefit as first-line treatment in patients with various solid tumor types.¹⁻³ The randomized phase III CheckMate 743 study demonstrated significant overall survival (OS) benefit with nivolumab plus ipilimumab versus chemotherapy in patients with unresectable pleural mesothelioma (PM; hazard ratio [HR], 0.74 [96.6% CI, 0.60 to 0.91] $P = .0020$).⁴ Subsequently, nivolumab plus ipilimumab was approved as a first-line treatment option for patients with unresectable PM in several countries, including the United States and the European Union.^{5,6}

Four-year follow-up data from CheckMate 743 showed sustained OS benefit with nivolumab plus ipilimumab versus chemotherapy (HR, 0.73 [95% CI, 0.61 to 0.87]).⁷ Here, we report 5-year clinical outcomes from CheckMate 743, the longest follow-up to date, to our knowledge, for first-line immunotherapy in PM. Additionally, we report exploratory biomarker analyses and treatment switching-adjusted

analyses in patients who received subsequent immunotherapy after first-line chemotherapy.

METHODS

The study design and patient eligibility criteria for CheckMate 743 are summarized in the Data Supplement (Fig S1, online only).⁴ Patients were randomly assigned 1:1 to receive nivolumab 3 mg/kg once every 2 weeks plus ipilimumab 1 mg/kg once every 6 weeks, or cisplatin 75 mg/m² or carboplatin area under the concentration-time curve 5 mg/mL per minute plus pemetrexed 500 mg/m² once every 3 weeks for a maximum of six cycles.

This trial was conducted in accordance with the Declaration of Helsinki and the International Conference on Harmonisation Good Clinical Practice Guidelines. The study protocol and amendments were approved by an institutional review board or independent ethics committee at each site. All patients provided written informed consent.

Assessments included 5-year OS, progression-free survival (PFS), objective response rate (ORR), and duration of response (DOR) in all randomly assigned patients and by tumor histology and safety. Additionally, we report OS and PFS by baseline monocytic myeloid-derived suppressor cell (M-MDSC) level and OS adjusted for treatment switching among patients in the chemotherapy arm. Additional methodology information is included in the Data Supplement.

RESULTS

Patients and Treatment Summary

Overall, 303 and 302 patients were randomly assigned to the nivolumab plus ipilimumab and chemotherapy arms, respectively; 300 (99%) and 284 (94%) patients received the respective study treatments (Fig 1).⁴ Baseline characteristics were similar across treatment arms (Table 1). Among randomly assigned patients, 6% of patients treated with nivolumab plus ipilimumab and 24% of patients treated with chemotherapy received subsequent immunotherapy (Data Supplement, Table S1).

Efficacy

At database lock (June 14, 2023; median follow-up for OS, 66.8 months), nivolumab plus ipilimumab demonstrated continued OS benefit versus chemotherapy (HR, 0.74 [95% CI, 0.62 to 0.88]); 5-year OS rates were 14% versus 6% (Fig 2A). Consistent OS benefit with nivolumab plus ipilimumab was observed across most subgroups (Fig 2B). Five-year OS rates with nivolumab plus ipilimumab versus

chemotherapy were 14% versus 8% in patients with epithelioid PM (HR, 0.85 [95% CI, 0.69 to 1.03]; Fig 2C) and 12% versus 1% in patients with nonepithelioid PM (HR, 0.48 [95% CI, 0.33 to 0.68]; Fig 2D).

Continued PFS improvement was observed with nivolumab plus ipilimumab versus chemotherapy in all randomly assigned patients (HR, 0.93 [95% CI, 0.77 to 1.13]); the 5-year PFS rate was 8% for nivolumab plus ipilimumab, whereas all patients in the chemotherapy arm were censored or experienced a PFS event before 5 years (Data Supplement, Fig S2A). PFS analyses by tumor histology are reported in the Data Supplement (Figs S2B and S2C).

ORRs were 39% with nivolumab plus ipilimumab versus 44% with chemotherapy in all randomly assigned patients (Data Supplement, Table S2). Of responders, DOR favored nivolumab plus ipilimumab over chemotherapy (Data Supplement, Fig S3A). At 5 years, 17% of responders treated with nivolumab plus ipilimumab versus 0% of responders treated with chemotherapy demonstrated ongoing response. Similar trends toward improved, sustained response with nivolumab plus ipilimumab versus chemotherapy were observed across tumor histology subgroups (Data Supplement, Figs S3B and S3C).

Biomarker Analyses

Of the randomly assigned patients, 242 (80%) patients treated with nivolumab plus ipilimumab and 218 (72%) patients treated with chemotherapy had evaluable blood M-MDSC measurements (Data Supplement, Table S3). In the M-MDSC-evaluable population, nivolumab plus ipilimumab

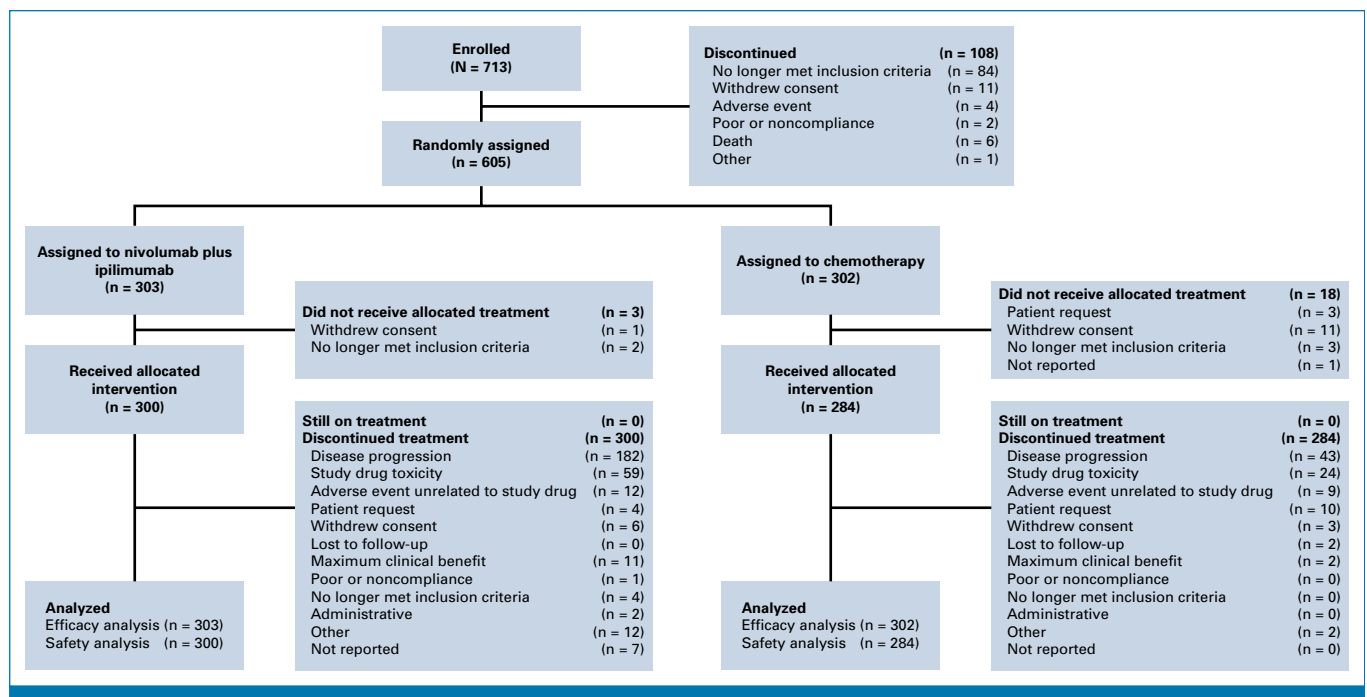


FIG 1. CONSORT diagram.

TABLE 1. Baseline Characteristics in All Randomly Assigned Patients and by Tumor Histology

Characteristic	All Randomly Assigned		Epithelioid		Nonepithelioid	
	Nivolumab Plus Ipilimumab (n = 303)	Chemotherapy (n = 302)	Nivolumab Plus Ipilimumab (n = 229)	Chemotherapy (n = 226)	Nivolumab Plus Ipilimumab (n = 74)	Chemotherapy (n = 76)
Age, years, median (IQR)	69 (65-75)	69 (62-75)	69 (64-75)	69 (62-74)	70 (67-74)	70 (64-76)
Sex, No. (%)						
Male	234 (77)	233 (77)	172 (75)	171 (76)	62 (84)	62 (82)
Female	69 (23)	29 (23)	57 (25)	55 (24)	12 (16)	14 (19)
Region, No. (%)						
North America	32 (11)	27 (9)	22 (10)	17 (8)	10 (14)	10 (13)
Europe	177 (58)	175 (58)	144 (63)	133 (59)	33 (45)	42 (55)
Asia	26 (9)	39 (13)	11 (5)	20 (9)	15 (20)	19 (25)
Rest of the world ^a	68 (22)	61 (20)	52 (23)	56 (25)	16 (22)	5 (7)
ECOG PS, ^b No. (%)						
0	114 (38)	128 (42)	92 (40)	106 (47)	22 (30)	22 (29)
1	189 (62)	173 (57)	137 (60)	120 (53)	52 (70)	53 (70)
Smoking status, No. (%)						
Current/former	173 (57)	171 (57)	126 (55)	122 (54)	47 (64)	49 (64)
Never	127 (42)	122 (40)	100 (44)	96 (42)	27 (36)	26 (34)
Unknown	3 (1)	9 (3)	3 (1)	8 (4)	0	1 (1)
Tumor histology, No. (%)						
Epithelioid	229 (76)	226 (75)	229 (100)	226 (100)	0	0
Nonepithelioid	74 (24)	76 (25)	0	0	74 (100)	76 (100)
Sarcomatoid	35 (12)	36 (12)	0	0	35 (47)	36 (47)
Mixed/other	39 (13)	39 (13)	0	0	39 (53)	40 (53)
Stage, No. (%)						
I	12 (4)	20 (7)	8 (4)	15 (7)	4 (5)	5 (7)
II	23 (8)	22 (7)	15 (7)	16 (7)	8 (11)	6 (8)
III	103 (34)	106 (35)	85 (37)	84 (37)	18 (24)	22 (29)
IV	160 (53)	149 (49)	116 (51)	107 (47)	44 (60)	42 (55)
Not reported	5 (2)	5 (2)	5 (2)	4 (2)	0	1 (1)

Abbreviation: ECOG PS, Eastern Cooperative Oncology Group performance status.

^aAustralia, Brazil, Chile, Colombia, Mexico, South Africa, and Turkey.

^bOn a score of 0 to 5, with higher scores indicating greater disability. One patient in the chemotherapy group had a baseline ECOG PS of 2 (protocol deviation).

improved OS versus chemotherapy (median [95% CI], 19.6 months [17.3 to 24.9] versus 14.9 months [12.3 to 16.5]; HR, 0.72 [95% CI, 0.59 to 0.88]; Fig 3A); PFS favored nivolumab plus ipilimumab over chemotherapy (Fig 3B). Among patients treated with nivolumab plus ipilimumab, higher M-MDSC levels were associated with worse OS and PFS outcomes in M-MDSC-evaluable patients (OS HR, 1.25 [95% CI, 1.09 to 1.43]; PFS HR, 1.23 [95% CI, 1.06 to 1.44]) and the epithelioid PM subgroup (OS HR, 1.27 [95% CI, 1.09 to 1.48]; PFS HR, 1.26 [95% CI, 1.06 to 1.50]; Fig 3C).

Treatment Switching–Adjusted Analyses

Of patients randomly assigned to the chemotherapy arm, 72 (24%) patients received subsequent immunotherapy. Adjusting for subsequent immunotherapy use in the chemotherapy arm using the inverse probability of censoring weighting (IPCW) method, median OS (95% CI) with

chemotherapy was 12.1 months (10.2 to 13.9), and the adjusted HR (95% CI) for nivolumab plus ipilimumab versus chemotherapy was 0.64 (0.53 to 0.78; Data Supplement, Fig S4A). Consistent results were observed in sensitivity analyses (Data Supplement, Fig S5). Treatment switching–adjusted analyses by tumor histology are reported in the Data Supplement (Supplemental Results; Figs S4B, S4C, S6, and S7).

Safety

Consistent with previous reports,^{4,8} no new safety signals were identified, as summarized in the Data Supplement (Tables S4–S6).

DISCUSSION

With a median follow-up of 66.8 months, nivolumab plus ipilimumab demonstrated long-term, durable survival and

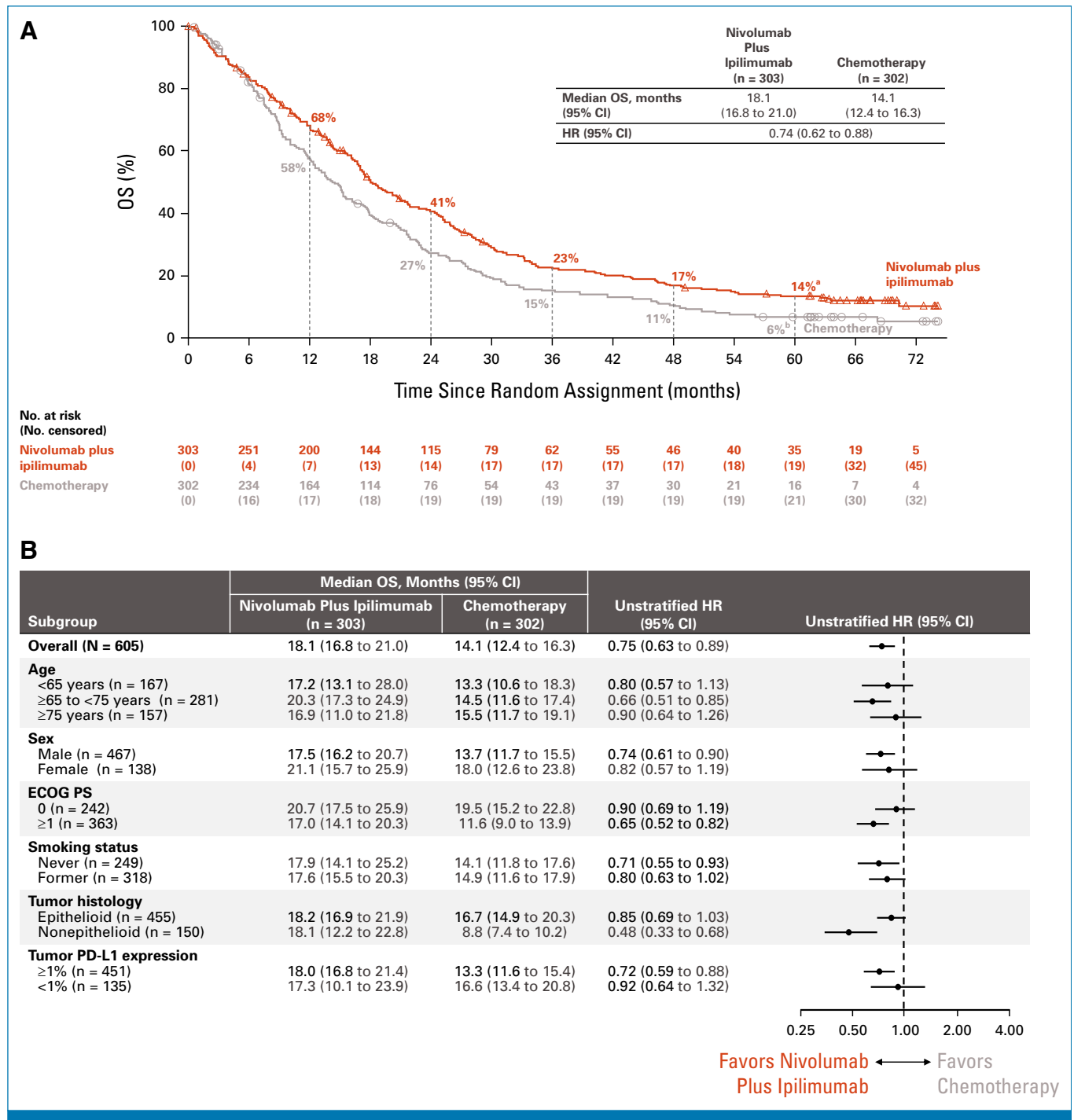


FIG 2. OS in (A) all randomly assigned patients, (B) key patient subgroups, (C) patients with epithelioid tumor histology, and (D) patients with nonepithelioid tumor histology. The 95% CIs for 5-year OS rates for all randomly assigned patients, patients with epithelioid PM, and patients with nonepithelioid PM in the nivolumab plus ipilimumab and chemotherapy arms were as follows: ^a10-18; ^b4-10; ^c10-19; ^d5-12; ^e6-22; and ^f0-7. ECOG PS, Eastern Cooperative Oncology Group performance status; HR, hazard ratio; OS, overall survival; PM, pleural mesothelioma. (continued on following page)

response benefit versus chemotherapy in all randomly assigned patients and across most subgroups. In patients with evaluable blood M-MDSC measurements, elevated baseline M-MDSC levels trended toward reduced benefit. After adjusting for treatment switching in the chemotherapy

arm, a greater magnitude in OS benefit with nivolumab plus ipilimumab was observed.

Improved survival and response outcomes with nivolumab plus ipilimumab were consistent with or better than other

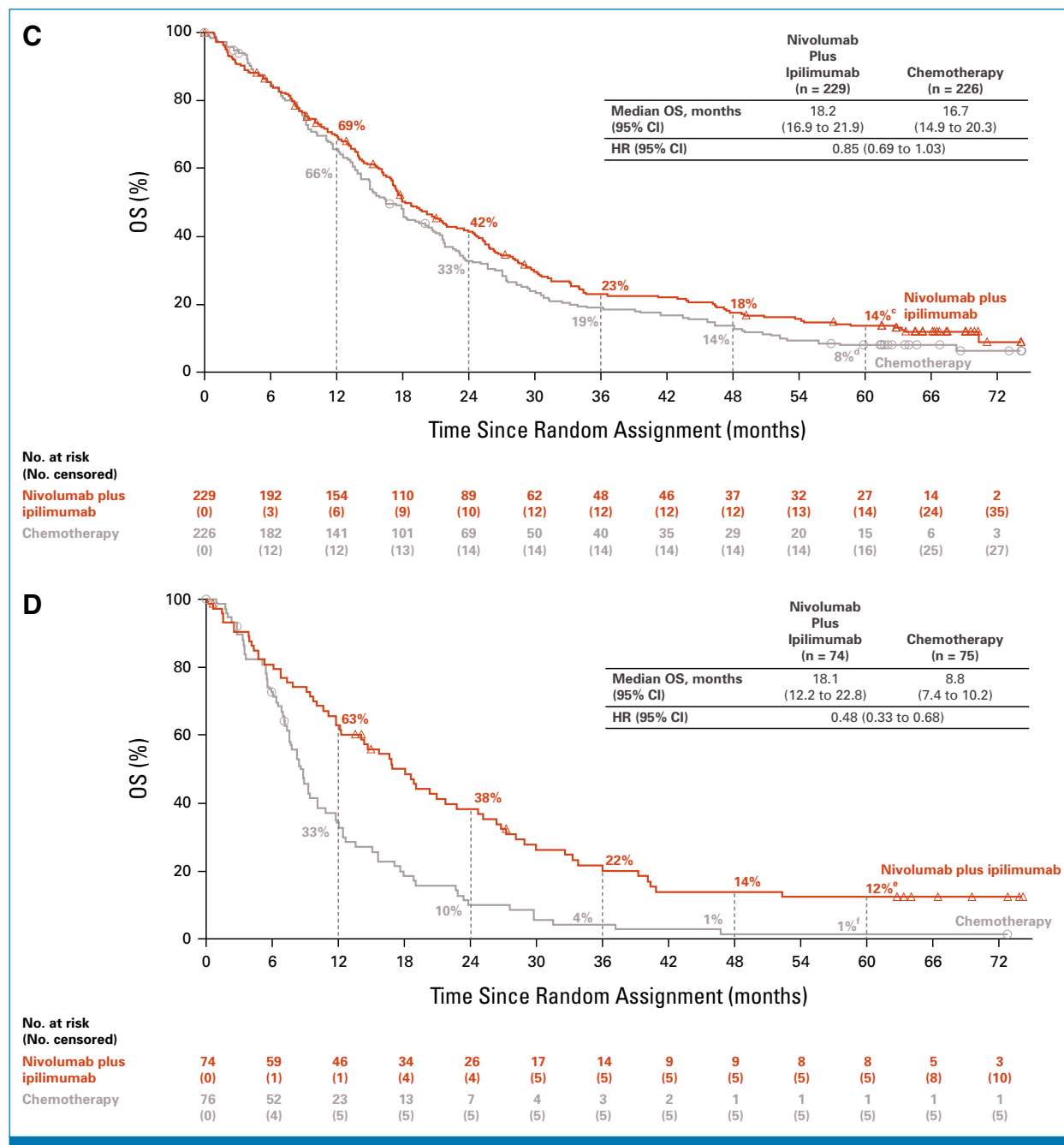


FIG 2. (Continued).

first-line immunotherapy-based treatments for unresectable PM.⁹⁻¹¹ In a meta-analysis comparing six randomized controlled trials of first-line treatments for PM, nivolumab plus ipilimumab significantly improved OS versus chemotherapy, similar to benefit with bevacizumab plus chemotherapy or pembrolizumab plus chemotherapy.¹² The magnitude of clinical benefit with nivolumab plus ipilimumab versus chemotherapy was greater in patients with non-epithelioid PM versus epithelioid PM in CheckMate 743, consistent with results for pembrolizumab plus chemotherapy versus chemotherapy in KEYNOTE-483, and may

have resulted from differences in immune-related gene expression across tumor histology subgroups, higher levels of tumor-infiltrating lymphocytes in non-epithelioid PM, and/or worse OS with chemotherapy in patients with non-epithelioid PM.^{13,14}

M-MDSCs have been associated with immunotherapy resistance and worse prognoses in patients with PM.¹⁵⁻²⁰ In CheckMate 743, OS and PFS with nivolumab plus ipilimumab were worse in patients with high M-MDSC levels in all randomly assigned patients and patients with epithelioid

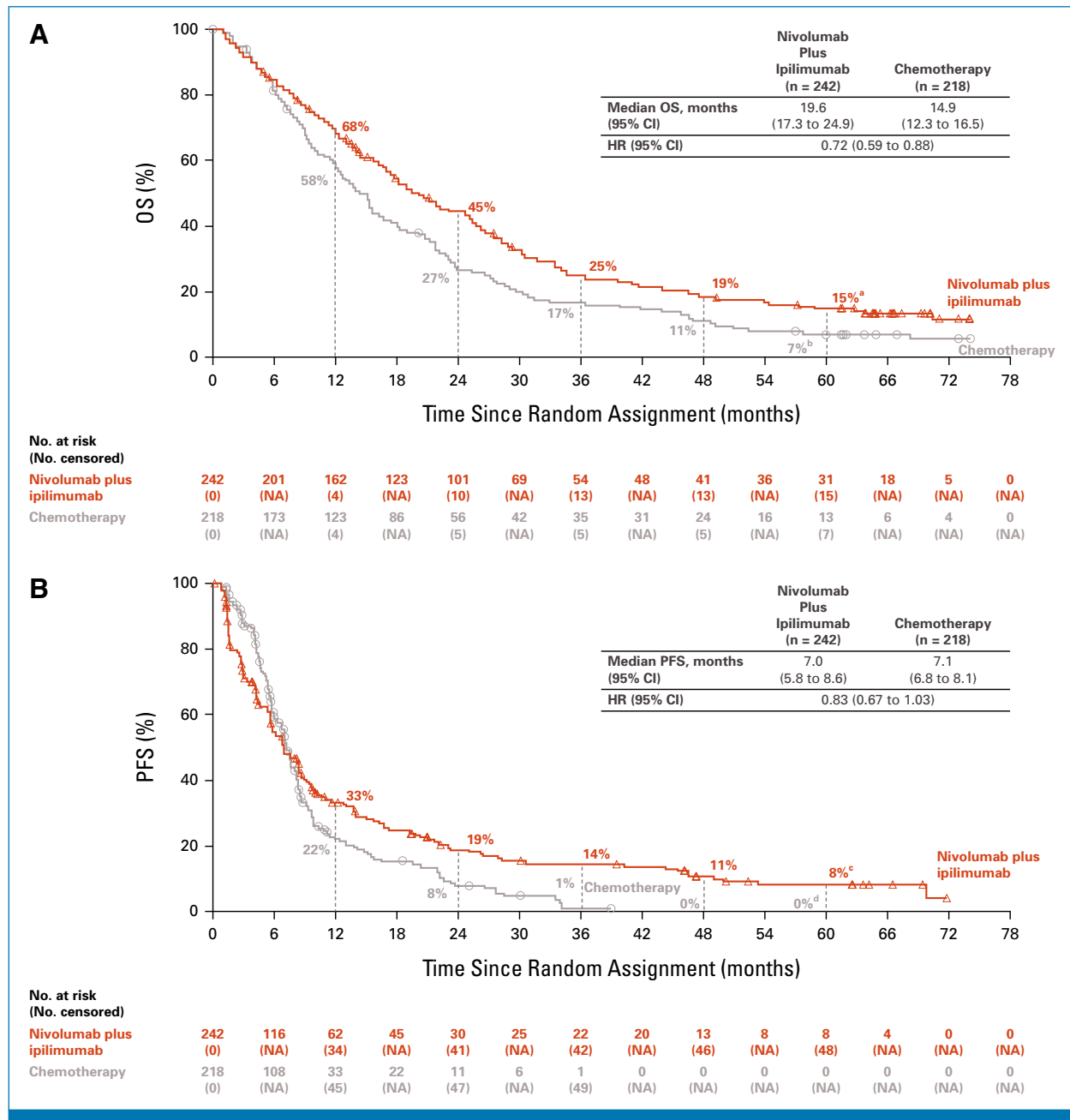


FIG 3. (A) OS and (B) PFS in the M-MDSC-evaluable population and (C) associations between blood M-MDSC with OS and PFS in the M-MDSC-evaluable population and tumor histology subgroups. The 95% CIs for 5-year OS and PFS rates in the nivolumab plus ipilimumab and chemotherapy arms were as follows: ^a11-20; ^b4-11; ^c4-13; ^dNA-NA. All subgroups included in the forest plot were predetermined at baseline before random assignment. HRs for each 1-unit increase in log-transformed blood M-MDSC levels were reported. An HR >1 indicated increased risk of an event with higher M-MDSC levels, whereas an HR <1 indicated decreased risk. HR, hazard ratio; M-MDSC, monocytic myeloid-derived suppressor cell; NA, not applicable/available; OS, overall survival; PFS, progression-free survival. (continued on following page)

PM; however, additional prospective studies are needed to confirm correlations between M-MDSC levels and clinical outcomes with first-line immunotherapy.

Patients with relapsing PM after first-line chemotherapy may benefit from second-line treatment.²¹⁻²⁴ The IPCW-adjusted median OS analysis in the chemotherapy group

decreased from 14.1 months to 12.1 months, amplifying the clinical benefit of nivolumab plus ipilimumab (HR adjusted from 0.75 to 0.64).

In conclusion, this 5-year update from CheckMate 743 demonstrated continued long-term, durable clinical benefit of first-line nivolumab plus ipilimumab in patients

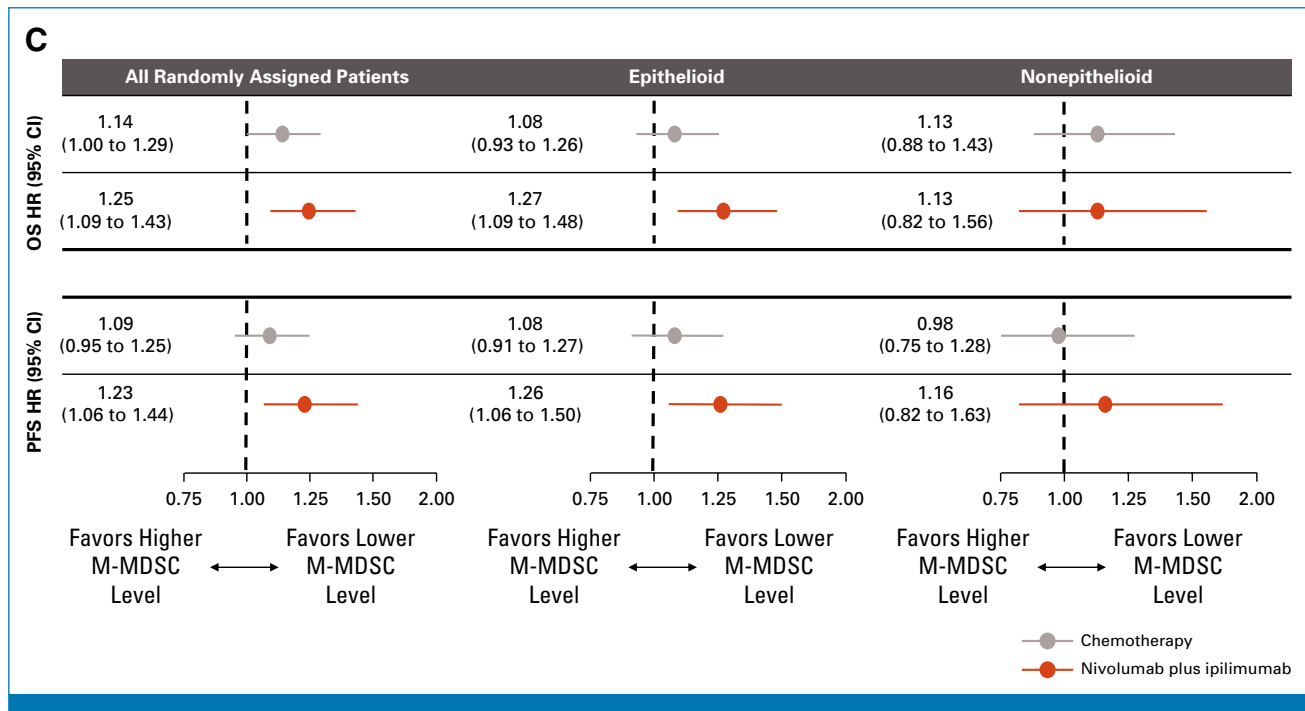


FIG 3. (Continued).

with PM, regardless of tumor histology. Future prospective analyses are needed to evaluate the prognostic or predictive value of M-MDSCs in patients with PM

treated with first-line immunotherapy and their role in the treatment selection process beyond current guidelines.²⁵

AFFILIATIONS

¹University of Lille, CHU Lille, INSERM U1189 OncoThAI, ONCOLille, Lille, France

²The Netherlands Cancer Institute and Leiden University Medical Center, Amsterdam, the Netherlands

³University of Western Australia, Perth, WA, Australia

⁴University of Texas MD Anderson Cancer Center, Houston, TX

⁵Okayama Rosai Hospital, Okayama, Japan

⁶Lausanne University Hospital, Lausanne, Switzerland

⁷Mayo Clinic, Rochester, MN

⁸The Royal Marsden Hospital, London, United Kingdom

⁹Centro Médico Nacional Siglo XXI, Mexico City, Mexico

¹⁰The Sunrise Centre, Royal Cornwall Hospitals NHS Trust, Truro, United Kingdom

¹¹Medical Oncology Division, IRCCS Policlinico San Martino, Genova, Italy

¹²Maria Skłodowska-Curie National Research Institute of Oncology, Warsaw, Poland

¹³Dicle University, Diyarbakir, Turkey

¹⁴Luis Carlos Sarmiento Angulo Cancer Treatment and Research Center (CTIC), Bogotá, Colombia

¹⁵Universidad El Bosque, Bogotá, Colombia

¹⁶Bristol Myers Squibb, Princeton, NJ

¹⁷Bristol Myers Squibb, Uxbridge, United Kingdom

¹⁸Université Paris Cité, Bichat-Claude Bernard Hospital, Thoracic Oncology Department, AP-HP. Nord Cancer Institute, Paris, France

CORRESPONDING AUTHOR

Arnaud Scherpereel, MD, PhD; e-mail: arnaud.scherpereel@chu-lille.fr.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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AUTHOR CONTRIBUTIONS

Conception and design: Arnaud Scherpereel, Paul Baas, Anne S. Tsao, Nobukazu Fujimoto, Solange Peters, Aaron S. Mansfield, Sanjay Popat, Muhammet A. Kaplan, Adam Lee, Virginia Ip, Gérard Zalcman

Administrative support: Yolanda Bautista Aragon, Raheela Soomro

Provision of study materials or patients: Paul Baas, Anna K. Nowak, Anne S. Tsao, Solange Peters, Sanjay Popat, Toby Talbot, Andrés Felipe Cardona, Gérard Zalcman

Collection and assembly of data: Arnaud Scherpereel, Paul Baas, Anna K. Nowak, Anne S. Tsao, Nobukazu Fujimoto, Aaron S. Mansfield, Sanjay Popat, Toby Talbot, Francesco Grossi, Dariusz Kowalski, Muhammet A. Kaplan, Andrés Felipe Cardona, Virginia Ip, Gérard Zalcman

Data analysis and interpretation: Arnaud Scherpereel, Paul Baas, Anna K. Nowak, Anne S. Tsao, Nobukazu Fujimoto, Solange Peters, Aaron S. Mansfield, Sanjay Popat, Yolanda Bautista Aragon, Francesco Grossi, Dariusz Kowalski, Muhammet A. Kaplan, Andrés Felipe Cardona,

Raheela Soomro, Nan Hu, Adam Lee, Virginia Ip, Yu-Han Hung, Gérard Zalcman

Manuscript writing: All authors

Final approval of manuscript: All authors

Accountable for all aspects of the work: All authors

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Five-Year Clinical Outcomes With Nivolumab Plus Ipilimumab Versus Chemotherapy as First-Line Treatment for Unresectable Pleural Mesothelioma in CheckMate 743

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Arnaud Scherpereel

Consulting or Advisory Role: MSD Oncology, Roche, Bristol Myers Squibb, AstraZeneca/MedImmune

Speakers' Bureau: AstraZeneca, Bristol Myers Squibb

Research Funding: Bristol Myers Squibb (Inst), MSD (Inst)

Travel, Accommodations, Expenses: Roche, AstraZeneca

Paul Baas

Honoraria: Bristol Myers Squibb, AstraZeneca (Inst), Fidia Farmaceutici S. p. A

Consulting or Advisory Role: Merck Sharp & Dohme (Inst), Bristol Myers Squibb (Inst)

Travel, Accommodations, Expenses: BMS (Inst)

Other Relationship: Bristol Myers Squibb

Anna K. Nowak

Consulting or Advisory Role: AstraZeneca, Douglas Pharmaceuticals

Research Funding: AstraZeneca (Inst)

Anne S. Tsao

Honoraria: UpToDate, AstraZeneca, BMS, Summit Pharmaceuticals

Consulting or Advisory Role: Genentech/Roche, Bristol Myers Squibb, AstraZeneca/MedImmune, Summit Pharmaceuticals, Novocure

Research Funding: Merck, Seagen, Boehringer Ingelheim, Bristol Myers Squibb, Genentech, Lilly

Patents, Royalties, Other Intellectual Property: UpToDate

Nobukazu Fujimoto

Honoraria: Chugai Pharma, Ono Pharmaceutical, Bristol Myers Squibb Japan, MSD, AstraZeneca

Consulting or Advisory Role: AstraZeneca

Research Funding: Ono Pharmaceutical (Inst), GlaxoSmithKline (Inst), AstraZeneca (Inst)

Solange Peters

Honoraria: Roche (Inst), Bristol Myers Squibb (Inst), Novartis (Inst), Pfizer (Inst), MSD (Inst), AstraZeneca (Inst), Takeda (Inst), Illumina (Inst), Medscape (Inst), Prime Oncology (Inst), RMEI Medical Education (Inst), Research to Practice (Inst), PER (Inst), Imedex (Inst), Ecancer (Inst), OncologyEducation (Inst), Fishawack Facilitate (Inst), PeerView (Inst), Medtoday (Inst), Mirati Therapeutics (Inst), Sanofi (Inst), Incyte (Inst), Seattle Genetics/Astellas (Inst), Ipsen (Inst), GlaxoSmithKline, Boehringer Ingelheim (Inst), Foundation Medicine (Inst)

Consulting or Advisory Role: Roche/Genentech (Inst), Novartis (Inst), Bristol Myers Squibb (Inst), Pfizer (Inst), MSD (Inst), Amgen (Inst), AstraZeneca (Inst), Janssen (Inst), Regeneron (Inst), Merck Serono (Inst), Boehringer Ingelheim (Inst), Takeda (Inst), Lilly (Inst), AbbVie (Inst), Bayer (Inst), Biocartis (Inst), Debiopharm Group (Inst), Illumina (Inst), PharmaMar (Inst), Sanofi (Inst), Seagen (Inst), Blueprint Medicines (Inst), Daiichi Sankyo (Inst), Incyte (Inst), Bioinvent (Inst), Clovis Oncology (Inst), Vaccibody (Inst), Foundation Medicine (Inst),

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Travel, Accommodations, Expenses: Roche, Bristol Myers Squibb, MSD, Sanofi, Incyte, MJH Life Sciences

Uncompensated Relationships: Annals of Oncology, ESMO, ETOP Scientific Chair, JTO Past Deputy Editor, SAMO President

Uncompensated Relationships: Oncosuisse President

Uncompensated Relationships: ESMO Open Associate Editor

Uncompensated Relationships: Swiss Cancer League Vice-President

Aaron S. Mansfield

Consulting or Advisory Role: AbbVie (Inst), Genzyme (Inst), Gilead Sciences (Inst), Abdera Therapeutics (Inst), Genprex (Inst), Orion (Inst), RayzeBio (Inst)

Research Funding: Bristol Myers Squibb (Inst)

Travel, Accommodations, Expenses: AbbVie, Roche

Other Relationship: Mesothelioma Applied Research Foundation, Friends of Patan Hospital Board of Directors

Sanjay Papat

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Consulting or Advisory Role: Boehringer Ingelheim, Roche, Pfizer, AstraZeneca, Bristol Myers Squibb, MSD, Guardant Health, Takeda, Bayer, Daiichi Sankyo, Janssen, GlaxoSmithKline, Lilly, Merck KGaA, Amgen, Pfizer, Pierre Fabre, Anheart Therapeutics, Arcus Biosciences, Ellipses Pharma, Erasca, Inc, Gilead Sciences, Pierre Fabre, PharmaMar, BioNTech SE, Genmab, IO Biotech, Nuvalent, Inc, SERVIER, Summit Therapeutics, Taiho Oncology, Bicycle Therapeutics

Research Funding: Boehringer Ingelheim (Inst), Epizyme (Inst), Bristol Myers Squibb (Inst), Clovis Oncology (Inst), Roche (Inst), Lilly (Inst), Takeda (Inst), Celgene (Inst), Novartis (Inst), ARIAD (Inst), MSD (Inst), Daiichi Sankyo (Inst), Guardant Health (Inst), Janssen (Inst), GlaxoSmithKline (Inst), Mirati Therapeutics (Inst), Trizell (Inst), Turning Point Therapeutics (Inst), Amgen (Inst), AstraZeneca (Inst)

Travel, Accommodations, Expenses: Roche/Genentech, Gilead Sciences

Toby Talbot

Honoraria: Bristol Myers Squibb Foundation, MSD, AstraZeneca, Regeneron, Pierre Fabre

Consulting or Advisory Role: Bristol Myers Squibb Foundation, MSD, Regeneron, Pierre Fabre

Travel, Accommodations, Expenses: MSD

Francesco Grossi

Consulting or Advisory Role: MSD Oncology, Bristol Myers Squibb, AstraZeneca, Roche, Pfizer, Bayer, Lilly, Novartis Italy, Sanofi, Accord Healthcare, BeiGene, Daiichi Sankyo/Astra Zeneca, Johnson & Johnson/Janssen, Italfarmaco, MEDIAN Technologies, Pierre Fabre, PharmaMar, Regeneron, Takeda

Speakers' Bureau: MSD Oncology, Bristol Myers Squibb, AstraZeneca, Roche, Pierre Fabre, Amgen, Celgene, Lilly, Pfizer, Sanofi, Accord Healthcare, GENTILI, Johnson & Johnson/Janssen, Merck, Novartis, Takeda

Research Funding: Bristol Myers Squibb

Travel, Accommodations, Expenses: Bristol Myers Squibb, MSD, Roche, AstraZeneca, Pierre Fabre, Celgene, Amgen, Lilly, Novartis

Dariusz Kowalski

Consulting or Advisory Role: Bristol Myers Squibb, Boehringer Ingelheim, Merck Serono, Roche/Genentech, AstraZeneca, MSD, Pfizer, Amgen, Johnson & Johnson/Janssen, Takeda, Sanofi/Aventis, Medison, Merck, Daiichi Sankyo/UCB Japan

Andrés Felipe Cardona

Leadership: FICMAC

Honoraria: Bristol Myers Squibb, Roche, Boehringer Ingelheim, AbbVie, Merck Sharp & Dohme, Celldex

Consulting or Advisory Role: Roche, Merck Sharp & Dohme, Novartis, AstraZeneca, Bristol Myers Squibb, Foundation Medicine, Boehringer

Ingelheim, Foundation for Clinical and Applied Cancer Research (FICMAC), Janssen

Speakers' Bureau: Merck Sharp & Dohme, Roche, Bristol Myers Squibb, Novartis, Foundation for Clinical and Applied Cancer Research (FICMAC), Foundation Medicine

Research Funding: AstraZeneca, MSD Oncology (Inst), Janssen

Travel, Accommodations, Expenses: Merck Sharp & Dohme, Bristol Myers Squibb, Roche, Boehringer Ingelheim, Foundation Medicine

Adam Lee

Employment: Bristol Myers Squibb/Celgene, Genmab

Stock and Other Ownership Interests: Bristol Myers Squibb/Celgene

Virginia Ip

Employment: Bristol Myers Squibb/Celgene

Stock and Other Ownership Interests: Bristol Myers Squibb/Celgene

Travel, Accommodations, Expenses: Bristol Myers Squibb/Celgene

Yu-Han Hung

Employment: Bristol Myers Squibb

Stock and Other Ownership Interests: Bristol Myers Squibb

Travel, Accommodations, Expenses: Bristol Myers Squibb

Gérard Zalcman

Honoraria: Roche, Lilly, Pfizer, Bristol Myers Squibb, AstraZeneca, Boehringer Ingelheim, MSD Oncology, Inventiva Pharma, Da Volterra
Consulting or Advisory Role: Roche (Inst), Boehringer Ingelheim (Inst), Pfizer (Inst), Bristol Myers Squibb, Bristol Myers Squibb (Inst), MSD Oncology (Inst), Inventiva Pharma (Inst), AstraZeneca (Inst), Da Volterra (Inst)

Research Funding: Roche (Inst), Pfizer (Inst), AstraZeneca (Inst), Bristol Myers Squibb (Inst), Roche (Inst), Takeda (Inst), Regeneron (Inst)

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ORIGINAL ARTICLE

Evaluation of an educational program for nurses on the care coordination of pleural mesothelioma patients: A pilot randomized controlled trial

Yasuko Nagamatsu¹  | Wakanako Ono² | Yumi Sakyo¹  |
Edward Barroga³ | Keiko Hosokawa⁴ | Minako Ito⁵ | Shino Matsuura⁶ |
Taiki Fukujin⁷ | Yoko Maehara⁸ | Masaya Ito⁹ | Haruhiko Mitsunaga¹⁰ |
Satomi Nakajima¹¹ | Nobukazu Fujimoto¹² | Kazunori Okabe⁸

¹St. Luke's International University, Tokyo, Japan

²Tokyo Metropolitan University, Tokyo, Japan

³Showa Medical University, Tokyo, Japan

⁴St. Luke's International Hospital, Tokyo, Japan

⁵Tokyo Medical University, Tokyo, Japan

⁶Toho University, Chiba, Japan

⁷Hyogo Medical University, Hyogo, Japan

⁸Bell Land General Hospital, Osaka, Japan

⁹National Center of Neurology and Psychiatry, Tokyo, Japan

¹⁰Nagoya University, Nagoya, Japan

¹¹Musashino University, Tokyo, Japan

¹²Okayama Rosai Hospital, Okayama, Japan

Correspondence

Yasuko Nagamatsu, Graduate School of Nursing Science, St. Luke's International University, 1Akashi-cho, Chuo-ku, Tokyo 104-0044, Japan.

Email: sarah-nagamatsu@slcn.ac.jp

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Abstract

Objective: This study aimed to evaluate the effects of the *care coordination of pleural mesothelioma program* (CCOM program), an educational program that we developed for nurses to improve their knowledge, attitude, and confidence on the care coordination of pleural mesothelioma (PM) patients.

Methods: In this randomized controlled study relative to the CCOM program, we measured the self-reported total scores of knowledge, attitude, and confidence of nurses before (pre-test) and after (post-test) the CCOM program. The CCOM program consisted of a care guide and nine study videos featuring various aspects of PM (2 h 15 min) and a face-to-face workshop (3 h 30 min). Sixty participants were randomly assigned to the intervention group ($n = 30$; with program) and control group ($n = 30$; without program). Fifty-eight participants completed the study (intervention group, 28; control group, 30). The total scores of knowledge, attitude, and confidence at pre-test and post-test were compared using one-way repeated measures analysis of variance (ANOVA).

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Results: At pre-test, the groups showed no significant differences in age, experience of taking care of PM patients, and working department, except for the years of experience as a nurse. The CCOM program improved the knowledge, attitude, and confidence in the intervention group, whereas the control group showed stability in the scores. Repeated measures ANOVA showed significantly greater beneficial changes in the intervention group on main measurement scores.

Conclusions: The CCOM program was effective in improving the knowledge, attitude, and confidence of nurses on the care coordination of PM patients.

KEYWORDS

ANOVA, care coordination, education, mesothelioma, nurse, randomized controlled trial

1 | INTRODUCTION

Pleural mesothelioma (PM) is a rare malignancy (Beasley et al., 2021) which is almost exclusively caused by asbestos exposure (van Zandwijk et al., 2022). The survival period after diagnosis is as short as 8–19 months (Asciak et al., 2021; Bou-Samra et al., 2023; Fournel et al., 2022; Guzmán-Casta et al., 2021; Hasegawa et al., 2024; Kang et al., 2021; Kerrigan et al., 2022). PM causes a series of debilitating symptoms (Nagamatsu et al., 2022a) and additional distress as a victim of the asbestos industry (Guglielmucci et al., 2018). The number of annual deaths from PM in Japan is about 1600, and this number has been increasing in recent years (Japan Ministry of Health, 2025). The scarcity of specialized PM practitioners in Japan often requires patients to travel domestically within Japan to receive appropriate treatment and management services. When these patients return to their own hometown in Japan, their treatment and care are often discontinued.

A previous study of bereaved family members reported that PM patients rarely achieved a good quality of life in the terminal stage at home, which is related to the failure of care provision (Nagamatsu et al., 2022a). This indicates that receiving quality care when a patient becomes critical is crucial at home for PM patients. However, it is very challenging to provide quality care in a timely manner to PM patients because of several reasons. Firstly, PM progresses rapidly. Secondly, care provided in acute care hospitals and in home-based settings operates independently and remains fragmented (i.e., fragmented care), which results in a lack of continuity. Care coordination is expected to improve the fragmentation of care and bring consistency, continuity, and coordination to care.

Fragmentation of care also hinders nurses in acute care hospitals from understanding the care needs of PM patients living at home after discharge, leading to failed care

coordination. Finally, there is a limited number of medical institutions providing treatment and care for PM. A meta-analysis previously reported that cancer care coordination improved 81% of the outcomes, including screening, measures of patient experiences with care, and quality of end-of-life care (Gorin et al., 2017). With the growing complexity of the needs of patients, nurses must play a substantial role in the care coordination of patients with complex care needs (Karam et al., 2021). However, previous reviews have reported on only some conditions for achieving successful interprofessional care coordination such as having a shared vision (Khatri et al., 2023), using guidelines (Lisy et al., 2021), frequent communication and clinical interaction (Khatri et al., 2023; Lisy et al., 2021), recognition of the skills of other professionals (Khatri et al., 2023), defining the roles of each health care provider (Lisy et al., 2021), providing referrals (Khatri et al., 2023), producing summaries (Khatri et al., 2023), and conducting trainings (Khatri et al., 2023; Lisy et al., 2021). To promote care coordination in PM care, a care guide was developed previously (Nagamatsu & Sakyo, 2023). However, to fully accelerate care coordination, it is necessary to share the care needs of patients and their families between hospitals and communities, as well as to confirm the individual roles of nurses in providing patient-centered care.

In this study, we developed and conducted an educational program for nurses on the care coordination of PM patients (i.e., CCOM program). Our primary aim is to evaluate the effects of the CCOM program on the knowledge, attitude, and confidence of nurses on the care coordination of PM patients.

2 | THEORETICAL FRAMEWORK

The theoretical model of self-efficacy by Bandura and Walters (1977) and Bandura (1986), which is based on

the social learning theory, was used as the framework for the present study. *Perceived self-efficacy* is concerned with people's beliefs in their capabilities to exercise control over their own functioning and over events that affect their lives (Bandura, 1994). People's beliefs in their efficacy are developed from four main sources of influence: *mastery experiences, witnessing people similar to oneself manage task demands successfully, social persuasion that one has the capabilities to succeed in given activities, and inferences from somatic and emotional states indicative of personal strengths and vulnerabilities* (Bandura, 1994). Multiple studies have reported the effectiveness of self-efficacy in nursing education (Arslan et al., 2018; Cayır & Ulupınar, 2021). Nurses face limited opportunities to care for PM patients with complex conditions. In the present study, we used Bandura et al.'s theoretical model of self-efficacy to implement an educational program to improve nurses' care coordination skills. The program provides opportunities to learn from and cooperate with other participants in the care coordination of PM patients.

3 | METHODS

3.1 | Study design

This study was an open-labeled randomized controlled trial (pre-test and post-test design) with two parallel groups at 1:1 ratio. The trial protocol was registered with the University Hospital Medical Information Network Clinical Trials Registry (UMIN-CTR). The Trial registration number is ID: UMIN000052220. We report the trial results following the CONSORT 2010 Statement (Shulz et al., 2010).

3.2 | Intervention

The intervention group received the CCOM program developed and previously pilot-tested by the research team (Nagamatsu et al., 2025). The intervention was developed using the instructional design method (Gagne et al., 2004) to solve the difficulties experienced by nurses in the care coordination of PM patients. Instructional design is a systematic process of planning and delivering instruction based on an understanding of how learning occurs through internal cognitive processes (Gagne et al., 2004). Steps and processes of instructional design enable educators to determine the scope of the course contents, sequence of instructions, innovative presentation, and evaluation strategies (Obizoba, 2015). Instructional design identifies instructional problems, goals, learners'

characteristics, content and context of instruction (Obizoba, 2015). Instructional design-driven programs improve nurses' knowledge, self-efficacy, confidence, attitude, and clinical practice (Jia et al., 2025; Kim et al., 2020; Chu et al., 2019). In terms of PM care, which is a rare disease, nurses were inexperienced and unable to fully understand the care needs of PM patients, particularly those in the terminal stage. Nurses were unclear as to the care plans that they should implement as well as their roles within the fragmented healthcare system.

As for designing the instructions of the educational program, we adopted the Dick and Carey systems approach model (Dick et al., 2021). The CCOM program for nurses on the care coordination of PM patients consisted of two stages: (1) preparation and (2) face-to-face workshop. The preparation stage consisted of nine study videos featuring various aspects of PM (15 min each video) and a reading care guide as handout. The contents of the videos (2 h and 15 min) and face-to-face workshop (3 h and 30 min) are shown in Table 1. The face-to-face workshop included a lecture by a home care nurse regarding the care needs of PM patients and their families in the terminal stage at home using actual PM cases. The control group received no education.

TABLE 1 Contents of the educational program for nurses on the care coordination of pleural mesothelioma patients.

Preparation of study videos reading care guide as handout (2 h 15 min)	
Asbestos and mesothelioma	15 min
Surgery	15 min
Anti-tumor therapy	15 min
Nursing care	15 min
Social service	15 min
Home nursing	15 min
Psychological support	15 min
Social benefits and peer support	15 min
Narrative of bereaved family of PM patient	15 min
Face-to-face workshop (3 hours 30 minutes)	
Introduction and ice-breaking	30 min
Lecture by home care nurse:	30 min
Care needs of PM patients and their families in the terminal stage at home	
Group work: Care coordination plan for terminal case	90 min
Presentation	30 min
Activity introduction of advocacy group	20 min
Certificate award ceremony	10 min

3.3 | Outcome measures

The three outcome measures [(1) Knowledge of disease and treatment of PM, (2) Attitude toward care coordination of PM patients, (3) Confidence in the care coordination of PM patients] were originally developed by the research team (Nagamatsu et al., 2025) based on the theoretical model of self-efficacy by Bandura and Walters (1977) which was used as the framework for the present study to implement the educational program to improve nurses' care coordination skills. The development of these outcome measures was one of the steps in the instructional design method used to develop the CCOM Program. The instructional design model by Dick et al. (2021) consists of 10 steps, and Step 5 focuses on developing assessment instruments that evaluate learners' achievement of instructional objectives. Accordingly, nurses' confidence was measured corresponding to the 10 instructional goals identified in Step 1.

Items for the outcome measure: **(1) Knowledge of disease and treatment of PM** (*Knowledge*) were developed to assess the information required for nurses to achieve those goals. Items for the outcome measure. **(2) Attitude toward care coordination of PM patients** (*Attitude*) were constructed based on the difficulties reported by nurses providing care for PM patients (Nagamatsu et al., 2012) and the challenges identified during the feasibility study of the care guide (Nagamatsu & Sakyo, 2023). Items for the outcome measure. **(3) Confidence in the care coordination of PM patients** (*Confidence*) were based on the difficulties encountered by the nurses in the care coordination of PM patients.

To ensure content relevance, four nurses with clinical experience in PM reviewed the outcome measures. Based on their feedback, necessary modifications were made, and a pilot test was conducted to confirm the clarity and applicability of these evaluation tools or indicators (Nagamatsu et al., 2025). To assure content relevance, four nurses with clinical experience in PM reviewed the content and based on their comments, the necessary modifications were made and pilot-tested (Nagamatsu et al., 2025).

The three outcome measures are detailed below:

(1) Knowledge of disease and treatment of PM (*Knowledge*): eight items (1 = right answer, 0 = wrong answer). The total score range is 0–8, and a higher score indicates higher knowledge. The knowledge scale consists of eight statements that measure knowledge in the following areas which are relevant to the care coordination of PM patients: (1) organ which develops mesothelioma, (2) pleurectomy-decortication, (3) symptom to control after extra pleural pneumonectomy,

(4) immune checkpoint inhibitors for PM, (5) occupations posing a risk of asbestos exposure, (6) benefit for PM caused by occupational exposure to asbestos, (7) asbestos health damage relief compensation system, and (8) side effects of immune checkpoint inhibitors in PM (Table 2). Participants select the answer(s) from the choices provided.

(2) Attitude toward care coordination of PM patients (*Attitude*): Original 10 items; 5-point Likert scale (1 = strongly agree, 5 = never agree). The total score range is 10–50, and a higher score indicates a positive attitude. The original items were developed based on the attitudes of nurses who had difficulties in the care coordination of PM patients (Table 3). This scale showed Cronbach's α coefficients of 0.706 at pre-test, 0.872 at post-test, and 0.873 for the whole study.

(3) Confidence in the care coordination of PM patients (*Confidence*): Original 10 items; 5-point Likert scale (1 = not confident, 5 = confident). The total score range is 10–50, and a higher score indicates a higher confidence. Items are based on the difficulties encountered by the nurses in the care coordination of PM patients (Table 4). This scale showed Cronbach's α coefficients of 0.905 at pre-test, 0.951 at post-test, and 0.938 for the whole study.

3.4 | Evaluation of the CCOM program

Appropriateness of the program implementation method, appropriateness of the program duration, and usefulness in caring for PM patients were assessed using a 5-point Likert scale (from agree to disagree). Additionally, participants were asked to provide open-ended comments about the CCOM program.

3.5 | Setting and participants

This study was conducted from November 2023 to December of 2023. The CCOM program for the intervention group was conducted three times in a university in Tokyo and once in a private hospital in Osaka from November 25 to December 16, 2023.

We carried out a purposive sampling of nurses who (i) have a nursing license and (ii) understand the Japanese language. After obtaining approval from our university review board, invitation forms were sent to the 24 hospitals that have an asbestos disease center.

The flow diagram for randomization is shown in Figure 1. A total of 60 nurses agreed to participate in this study. Of these 60 nurses, two in the intervention group could not attend the face-to-face workshop because of

TABLE 2 Outcome measure: Knowledge of disease and treatment of PM.

1.	Choose the organ which does not develop mesothelioma a. Peritoneum b. Testicular serosa c. Pericardium d. Fascia
2.	Choose the correct description below about pleurectomy-decortication a. Radiation therapy is often given after surgery b. It is a radical treatment for mesothelioma c. Less likely to cause atrial fibrillation than extrapleural pneumonectomy
3.	Choose the most important symptom that needs to be controlled after extrapleural pneumonectomy a. Hypoglycemia b. Constipation c. Loss of appetite
4.	Choose two immune checkpoint inhibitors approved for the treatment of PM a. Nivolumab b. Pembrolizumab c. Avelumab d. Ipilimumab
5.	Which occupations pose a risk of exposure to asbestos? a. Electrician b. Teacher c. Construction worker d. All of the above
6.	Choose the correct statement regarding PM caused by occupational exposure to asbestos a. Eligible for asbestos health damage relief compensation b. Workers' compensation can only be applied within 10 years after retirement c. Not eligible to apply for workers' compensation if one has been informed of the dangers of asbestos
7.	Which of the following statement is incorrect regarding the asbestos health damage relief compensation system? a. Applications can be made even after the patient's death b. You can receive almost the same compensation as workers' compensation c. Can apply for compensation at the public health center d. Patients with asbestos-related lung cancer can also apply for compensation
8.	Choose the correct description about the side effects of immune checkpoint inhibitors in PM a. Frequent dermatitis b. To ensure effectiveness of the therapy, it is better to tolerate the effects as much as possible c. Steroids are contraindicated because the intestines and skin become hypersensitive

work duty in their current workplace. Thus, a total of 58 nurses were evaluated.

3.6 | Data collection

The participants initially completed a self-administered questionnaire documenting their age, years of experience

as a nurse, number of PM patients taken care of, and current work department during the time of their application to the educational program. Thereafter, the participants completed a self-administered questionnaire of primary outcome at the pre-test and post-test. The intervention group answered at the post-test form at the end of the education program, and the control group answered the post-test 2 weeks after the pre-test.

TABLE 3 Outcome measure: Attitude toward care coordination of PM patients.

1	Pain in the terminal stage of PM cannot be controlled
2	It is difficult to ask PM patients and their families the intent for end-of-life care
3	I feel hesitant to contact a local medical facility and consult it about discharged PM patients
4	I am too busy to provide psychological care to PM patients and their family
5	I have no one to ask about PM care
6	It is a burden to suggest home care or palliative care to discharging PM patients
7	It is not the role of nurses to suggest about social benefits
8	It is not the role of nurses to introduce a patient support group
9	I cannot sympathize with PM patients and their family as regards their anger against asbestos
10	I do not know how to handle the victim feeling of PM patients

Note: 5-point Likert scale (1 = strongly agree, 5 = never agree).

TABLE 4 Outcome measure: Confidence in the care coordination of PM patients.

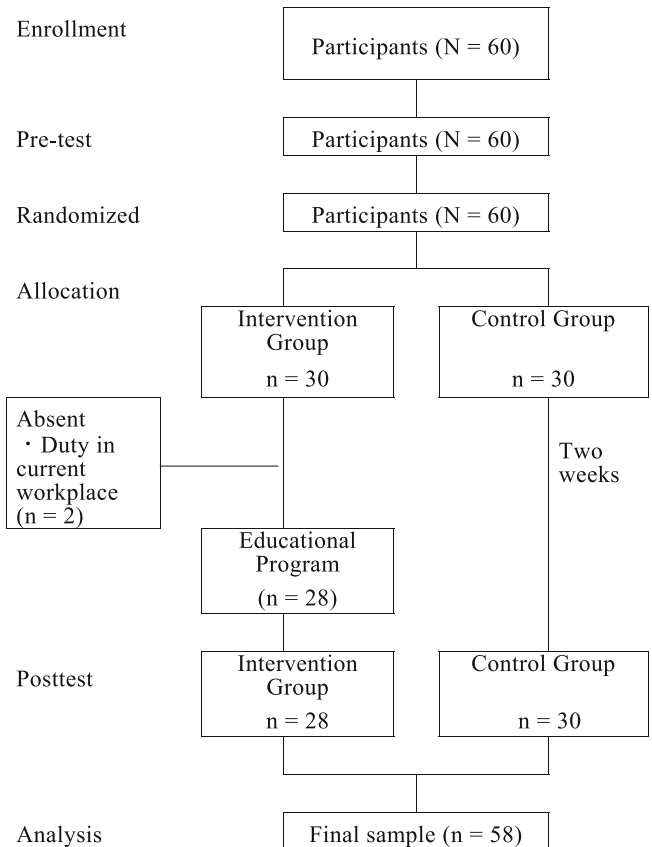
1	To interview newly diagnosed PM patients to check their understanding of the disease and treatment
2	To suggest social benefits to PM patients
3	To provide information about a patient support group
4	To provide care to manage the effects of surgery
5	To assess and manage the side effects of anti-tumor therapy
6	To connect local care for discharging PM patients
7	To suggest home care or palliative care to discharging PM patients
8	To ask PM patients where they want to die
9	To suggest grief care to the family
10	To show empathy to the victim's feeling

Note: 5-point Likert scale (1 = not confident, 5 = confident).

Reminder mails were sent to the control group. Data were collected from the end of November 2023 to the middle of December 2023.

3.7 | Sample size

The sample size for this study was calculated from the effects measured from a pilot study conducted by the research team (Nagamatsu et al., 2025). The pilot study was a single arm study (intervention group only)

**FIGURE 1** Flow diagram of randomization.

and 31 nurses answered questionnaire before, after, and 1 month later after the educational program. The sample size for this study was calculated with a significance level of .05, a power of 95%, and an effect size of 0.796 (attitude) which was the smallest among three measures. It was determined that 10 nurses or 5 per group would be required using G*Power software version 3.1 (Kang, 2021). To accommodate potential losses and because there were many nurses who wanted to participate in the CCOM program, the study enrolled a total of 60 nurses.

3.8 | Allocation

Participants were randomly assigned in a 1:1 ratio to either the intervention or control group using random permuted block sizes of 4. The two groups were assigned so that there was no difference in the proportion of those with or without experience of care of PM. The randomization sequence was generated by the principal investigator (corresponding author) using the cloud service Mujinwari (<https://autoassign.mujinwari.biz/>).

3.9 | Blinding

Participants were not blinded to the group allocation and intervention. All assessments were self-reported and data analysis was blinded to the group allocation. Participants did not know which group they will be assigned to at the time of giving their consent for participation.

3.10 | Statistical analysis

The distributions of the sociodemographic characteristics of the participants, such as age, years of clinical experience, and number of PM patients taken care of, were described and expressed as mean $\pm$ standard deviation. The participants' current workplaces (e.g., palliative care unit, pulmonary ward) were also described. A difference was considered significant when the corresponding p value was $< .05$. The distribution of all variables was checked for normality; nonparametric statistics were used when appropriate. The characteristics of the participants were compared between groups using the chi-square test for categorical variables and the t -test for continuous variables. Repeated measures multivariate analysis of variance was used to compare the total scores of the primary outcomes according to group, time, and group*time interaction. In the present study, "time" refers to the number of the test; that is, the pre-test and the post-test, not the actual time between the two tests. Data were analyzed using SPSS version 27.0.

3.11 | Program evaluation analysis

Descriptive statistics were used to summarize the participants' quantitative ratings of the CCOM program. Inductive content analysis was conducted to analyze comments of open-ended questions in the post-test (Elo & Kyngäs, 2008). Three researchers who have experience of qualitative analysis independently read the comments and provided explanatory encoding for every sentence or paragraph related to the evaluation of the program. Then, each researcher generated categories and subcategories based on the similarity of each code. Thereafter, all researchers discussed the process for generating categories to enhance the trustworthiness of data analysis.

3.12 | Ethical consideration

This study observed all standards for the protection of human subjects as set forth by the Declaration of Helsinki. The participants were informed that they could

voluntarily stop their participation at any time without any questions or repercussions. The anonymity of their responses was also assured. Ethical approval for the study was obtained from the Research Ethics Committee of our university (approval number: 23-A074).

4 | RESULTS

4.1 | Sociodemographic characteristics

We recruited 60 participants and randomly assigned them to two groups. All the participants completed the pre-test and 58 completed the post-test (Figure 1). The characteristics of the participants in the intervention and control groups are shown in Table 5. There were no significant differences in the means of the age and number of PM patients taken care of. However, the control group had a significantly longer nursing experience than the intervention group ($p = .003$). Nursing experience was adjusted as a covariate in two-way repeated analysis of variance (ANOVA).

4.2 | Impact of the CCOM program on nurse' knowledge, attitude, and confidence

A comparison of the mean scores of knowledge, attitude, and confidence between the intervention and control groups, as well as the results of the two-way repeated-measures ANOVA with covariate adjustment (nursing experience) are shown in Table 6.

4.2.1 | Knowledge

The mean knowledge scores were not significantly different between the two groups at baseline; however, the intervention group showed significantly higher scores than the control group at the post-test. The results of the two-way repeated measures ANOVA test showed a significant large-size effect of time on the participants' mean knowledge scores, indicating a significant change between the pre-test and the post-test ($p < .001$, $\eta^2 = .37$) across the two groups. The main effect of the educational program was significant ($p < .001$, $\eta^2 = .42$). There was a significant interaction between group and time ($p < .001$, $\eta^2 = 0.51$).

4.2.2 | Attitude

The mean attitude scores were not significantly different between the two groups at baseline; however, the

TABLE 5 Characteristics of participants in the intervention and control groups.

	Intervention group		Control group		<i>p</i> value
	<i>n</i> = 28		<i>n</i> = 30		
	Mean	SD	Mean	SD	
Age	39.1	10.8	37.9	9.9	<i>p</i> = .710
Nursing experience (year ^a)	8.9	6.9	12.9	0.2	<i>p</i> = .003
Number of MP patient taken care of	<i>n</i>	(%)	<i>n</i>	(%)	<i>p</i> = .844
0	17		17		
1–5	8		10		
6–10	1		2		
11<	2		1		
Working department					<i>p</i> = .963
Ward	11		8		
Home care/Community nursing	5		6		
Outpatient department	2		4		
Chemotherapy department	2		3		
Nursing administration	2		1		
ICU	1		2		
Palliative care	1		1		
Clinical trial	1		1		
OR	0		1		
Others (master student, faculty, midwife, consultant)	3		3		
Mean scores on measurements (pre-test)					
Knowledge	3.7	1.6	3.5	1.4	<i>p</i> = .591
Attitude	32.6	4.2	31.4	5.6	<i>p</i> = .372
Confidence	23.4	7.9	20.8	8.2	<i>p</i> = .222

^aThere was a significant difference in nursing experience years between the intervention and control groups.

TABLE 6 Confidence, attitude, and knowledge by group over time.

	Intervention group (<i>n</i> = 28)		Control group (<i>n</i> = 30)		Two-way analysis of variance			
	Mean (SD)		Mean (SD)					
	Baseline	Post-test	Baseline	Post-test		Groups	Time	Group × Time
Knowledge ^a	3.7 (1.6)	7.3 (0.9)	3.5 (1.4)	3.8 (1.6)	<i>F</i> -value	39.40	32.05	58.02
					<i>p</i>	<.001	<.001	<.001
					η ²	0.42	0.37	0.51
Attitude ^a	32.6 (4.2)	40.0 (5.9)	31.4 (5.6)	31.9 (6.0)	<i>F</i> -value	15.70	14.82	26.18
					<i>p</i>	<.001	<.001	<.001
					η ²	0.22	0.21	0.32
Confidence ^a	23.4 (7.9)	40.3 (5.9)	20.8 (8.2)	24.3 (8.0)	<i>F</i> -value	43.57	51.99	41.51
					<i>p</i>	<.001	<.001	<.001
					η ²	0.44	0.49	0.43

^aYears of nursing experience were included as an adjusted covariate.

TABLE 7 Categories and subcategories identified from the qualitative content analysis of evaluation comments.

Learning care needs of PM patients and families	Cause, rarity, and fatality of the disease Specificity of surgery and postoperative sequelae Understanding applicable pharmacotherapy Rapid disease progression at home Psychological burden of patients and families
Empathizing PM patients and families	Power of family's narrative Recognizing that universal risk of exposed to asbestos
Efficiency of face-to-face workshop followed by preparation	Time efficiency of preparation with handouts and videos Learning from other participants' experiences Integrating knowledge into care through group work
Gaining support to perform care coordination	Acquainting other professionals and patient support group Meeting nurses to consult when facing care challenges

intervention group showed significantly higher scores than the control group at the post-test. The results of the two-way repeated measures ANOVA test showed a significant size effect of time on the participants' mean confidence scores, indicating a significant change between the pre-test and the post-test ($p < .001$, $\eta^2 = .21$) across the two groups. The main effect of the educational program was significant ($p < .001$, $\eta^2 = .22$). There was a significant interaction between group and time ($p < .001$, $\eta^2 = 0.32$).

4.2.3 | Confidence

The mean attitude scores were not significantly different between the two groups at baseline; however, the intervention group showed significantly higher scores than the control group at the post-test. The results of the two-way repeated measures ANOVA test showed a significant large-size effect of time on the participants' mean confidence scores, indicating a significant change between the pre-test and post-test ($p < .001$, $\eta^2 = 0.49$) across the two groups. The main effect of the educational program was significant ($p < .001$, $\eta^2 = 0.44$). There was significant interaction between group and time ($p < .001$, $\eta^2 = 0.43$).

4.3 | Evaluation of CCOM program

4.3.1 | Quantitative evaluation of the CCOM program

The participants rated the CCOM program as appropriate in its implementation (96.3%) and duration (93.3%), and useful in caring for PM patients and their families (96.7%).

4.3.2 | Qualitative content analysis of evaluation comments

The results of the qualitative content analysis are shown in Table 7. The comments from the participants were classified into four categories: "Learning care needs of PM patients and families," "Empathizing with PM patients and families," "Efficiency of face-to-face workshop followed by preparation," and "Gaining support to perform care coordination." Participants gained understanding of the unique characteristics of PM, including its cause, rarity, fatality, specific treatments, and the psychological burden experienced by the patients and their families. Moreover, the CCOM program promoted the ability to empathize from the powerful narratives by family members and awareness of the universal risk of exposure to asbestos. Additionally, the participants reported that the CCOM program, which consisted of preparation and face-to-face workshop, minimized the time commitment. The group work also provided an opportunity to learn from other participants and to integrate knowledge to actual care. Furthermore, the participants gained support in conducting care coordination from other professionals and patient support groups, as well as from other nurses when facing care challenges.

5 | DISCUSSION

5.1 | Effectiveness of the CCOM program

For mesothelioma care, reports of care fragmentation remain limited. Warnock et al. (2019) reported that fragmented care increased anxiety in Great Britain. In Japan, PM patients who could not access care when their condition became critical at home were at a higher risk of not achieving a good death (Nagamatsu et al, 2022b) and their bereaved family members suffered complicated grief (Nagamatsu et al., 2022a). Few studies have addressed the barriers to the care coordination of mesothelioma in Japan and other countries. Previous studies have shown the following barriers to care coordination: fragmentation

of the health service (Barrios et al., 2022; Klabunde et al., 2017); lack of experience of health care providers; lack of defined providers' roles (Hohmann et al., 2020); lack of a clear shared-care plan (Hohmann et al., 2020); inadequate communication between a specialist and a primary caregiver (Walsh et al., 2010); reduced personnel (Barrios et al., 2022); lack of a comprehensive information sharing and communication system (Hohmann et al., 2020). The CCOM program was effective in improving the participants' knowledge, attitude, and confidence as the educational program was designed to show the roles of nurses using an actual case of a PM patient at the terminal stage, providing a care guide for PM patients to make up for inexperience, and providing opportunity to develop a support system with other nurses which empowers nurses to overcome various barriers.

The social learning theory by Bandura and Walters (1977) proposed that learning was possible not only by personal experience, but also by observing the actions and results of others. As nurses (1) have limited opportunities to experience providing care for patients with rare malignancies such as PM, and (2) even more rarely provide care for PM patients in the terminal stage at home, it is difficult to learn the care needs of PM patients in the terminal stage at home from their own experience. The CCOM program provided opportunities for the nurses to learn the care needs of PM patients and their families in the terminal stage from the perspectives of home care nurses. The evaluation comments indicated that the face-to-face workshop, preceded by preparation, enabled the participants to understand the care needs of PM patients at home, as well as the necessary care to meet those needs, and explore strategies for care coordination. Moreover, the collaborative efforts in care planning were significant, viewing them as a valuable platform for exchanging expertise and experiences related to care coordination. This process also facilitates the development of a support network, which is consistent with the principles outlined in Bandura's social learning Theory, thus emphasizing observational learning and modeling within a social context. All these components underlie the effectiveness of this educational program in improving the confidence of the nurses.

5.2 | Limitations and future research

The effectiveness of the CCOM program was assessed only at two measurement points: pre-test and post-test. Future studies should include follow-up assessments after a certain period to examine the sustained effects of the program.

In the current educational program, we could not obtain the contents of communication skills in the case of dying PM patients and their families because we needed to prioritize the understanding of their care needs in the terminal stage at home and the necessary care coordination to meet these care needs. However, communication skills are essential to the practice of care coordination (Khatri et al., 2023; Lisy et al., 2021; Walsh et al., 2010). Therefore, the development of educational programs that equally focus on advanced communication skills are essential.

The outcome measures examined in the pilot study demonstrated only internal consistency and content validity. Concurrent validity against an external criterion was not assessed. Future studies should evaluate criterion-related validity and test-retest reliability and confirm the factor structure of these measures.

Finally, even if this educational program could improve the knowledge, attitudes, and confidence of the participating nurses, effective care coordination requires the ability to integrate all these three elements into care practice. The true effectiveness of an educational program must be evaluated based on the participants' future care coordination practices and the care satisfaction of PM patients and their families.

6 | CONCLUSION

We conducted a randomized controlled trial to evaluate an educational program for nurses on the care coordination of PM patients (i.e., CCOM program). The results showed that the educational program for nurses significantly improved their knowledge, attitude, and confidence in the intervention group compared with the nurses in the control group without the educational program.

AUTHOR CONTRIBUTIONS

Yasuko Nagamatsu had primary responsibility for conducting the study. **Nagamatsu** contributed to the conceptualization and study design, development and implementation of the educational program, data collection and analysis, drafting the initial version of the manuscript, and acquisition of funding. **Wakanako Ono and Keiko Hosokawa** contributed to the development of the educational program and conducted the qualitative analysis. **Shino Matsuura and Taiki Fukujin** contributed to the development and implementation of the educational program. **Yumi Sakyō** contributed to the qualitative analysis, data interpretation and critical revision of the manuscript. **Minako Ito** contributed to data collection, statistical analysis, and critical revision of the

manuscript. **Masaya Ito and Satomi Nakajima** contributed to the development of the educational program and data interpretation. **Haruhiko Mitsunaga** contributed to the conceptualization and study design, as well as statistical analysis. **Edward Barroga** contributed to English editing and critical revision of the manuscript. **Yoko Maehara, Nobukazu Fujimoto, and Kazunori Okabe** contributed to the development of the educational program and supervised the study. All authors read and approved the final manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interest.

ORCID

Yasuko Nagamatsu  <https://orcid.org/0000-0001-6797-8052>

Yumi Sakyo  <https://orcid.org/0000-0001-9519-5792>

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