P3.061-175: Intrapleural ^{211}At-Labeled Anti-CD44v6 VHH Dimer for TAM-Targeted α-Therapy in MPE

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IASLC 2026 World Conference on Lung Cancer
SEPTEMBER 12 – 15, 2026
SEOUL, REPUBLIC OF KOREA

INTRODUCTION

Malignant pleural effusion (MPE) confers poor prognosis for lung adenocarcinoma patients. SPP1$^+$/CD44v6$^+$ tumor-associated macrophages drive immunosuppression and harbor defective DNA-damage repair. We generated an α-particle radiotherapeutic agent ^{211}At-VHH targeting CD44v6. This study aims to investigate whether intrapleural ^{211}At-VHH eliminates pathological TAMs and reinvigorates anti-tumor immunity for MPE treatment.

METHODS

Human samples Public MPE scRNA-seq (GSE131907); primary human MPE for ex-vivo validation.

In-vitro Wild-type RAW264.7 were IL-4-polarized to detect endogenous CD44/CD44v6. Lentiviral-overexpressing and CRISPR-knockout RAW264.7 tool cells were built for subsequent targeting tests. CD44v6-targeted ^{211}At-VHH was prepared. Planned assays: binding, internalization, cytotoxicity, DNA-damage and ICD detection. Primary mouse TAMs will be FACS-sorted from MPE.

In-vivo MPE model will be established by intrapleural LLC-luc injection in C57BL/6 mice. Mice will receive intrapleural PBS, cold-VHH, free-^{211}At, non-targeted and targeted ^{211}At-VHH. Tumor burden, effusion and survival will be assessed by BLI and gross observation. Immune profiles will be analyzed by flow cytometry, western blot, IHC and multiplex-IF. CD8$^+$ T-cell depletion will confirm immune-dependent efficacy.

DISCUSSION AND CONCLUSION

Actionable Take-away Message SPP1$^+$/CD44v6$^+$ TAMs are therapeutically actionable vulnerabilities in lung adenocarcinoma malignant pleural effusion. Intrapleural CD44v6-targeted ^{211}At-VHH α-particle radioimmunotherapy eliminates immunosuppressive TAMs, ignites cGAS-STING-dependent anti-tumor immunity, representing a promising therapeutic modality for MPE.

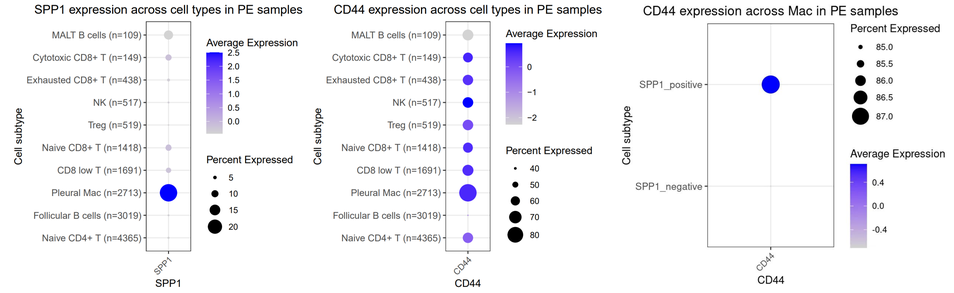
Future Direction for Research Further in-vitro assays will characterize ^{211}At-VHH targeting, cytotoxicity and ICD induction. In-vivo studies will test intrapleural ^{211}At-VHH therapeutic efficacy and cGAS-STING-related immune mechanisms in MPE mouse model. CD8$^+$ T-cell dependency will be validated. Human primary MPE samples will be applied for translational validation.

ACKNOWLEDGEMENTS

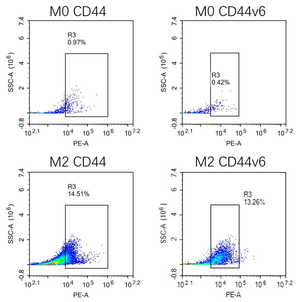
This study was supported by the National Natural Science Foundation of China (No. 82573498, 82403438), Sichuan Science and Technology Program (No. 2026NSFSC1944), the Noncommunicable Chronic Diseases-National Science and Technology Major Project (No. 2023ZD050610X/2023ZD0506100) and grant (No. RHM24211) from 135 Project of State Key Laboratory of Respiratory Health and Multimorbidity, West China Hospital, Sichuan University, "Qimingxing" Research Fund for Young Talents (HXQMX0212), and Noncommunicable Chronic Diseases-National Science and Technology Major Project (No. 2024ZD0520000 and 2024ZD0520006).

RESULTS

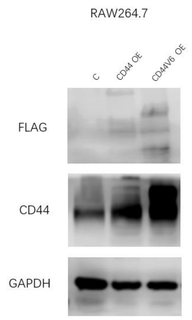
1. SPP1 and CD44 are predominantly expressed in pleural macrophages scRNA-seq of human MPE showed that among all immune cell subsets, pleural macrophages (n=2713) exhibited the highest SPP1 and CD44 expression. Within macrophage populations, CD44 was selectively enriched in SPP1-positive cells (~85-87% expressed), confirming the existence of a distinct SPP1$^+$/CD44$^+$ TAM subpopulation.



2. Endogenous CD44 and CD44v6 are up-regulated upon M2-polarization in wild-type RAW264.7 Flow cytometry using wild-type RAW264.7 macrophages showed M2 polarization markedly increased endogenous CD44$^+$ (M0 0.97% → M2 14.51%) and CD44v6$^+$ (M0 0.42% → M2 13.26%) cell fractions. These data demonstrate that pro-tumor M2-like polarization drives physiological upregulation of CD44/CD44v6, supporting CD44v6 as a relevant therapeutic target.



3. Lentiviral CD44/CD44v6 overexpression maintains native dimeric conformation Separate lentiviral transductions generated CD44- and CD44v6-overexpressing RAW264.7 tool cells for subsequent targeting assays. Membrane-protein western blot validated successful overexpression. A prominent dimeric band (~2-fold monomer molecular weight) was observed under non-denaturing (37 °C) conditions, confirming preserved native protein conformation.



Working model Calprotectin drives an autocrine RAGE/TLR4-NF-κB/STAT3 signaling loop in TAMs to upregulate SPP1 and CD44, establishing a pro-tumor phenotype with impaired DNA-damage repair. Intrapleural CD44-targeted ^{211}At α-therapy selectively eliminates these vulnerable TAMs, induces immunogenic cell death and dsDNA release, and hyperactivates cGAS-STING-driven anti-tumor immunity.

