Phosphatase-Excluding Polymer Micropatches for Enhancing Cytotoxic T-Lymphocytes-Based Cancer Therapy

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Abstract

Despite decades of progress in cytotoxic T Lymphocyte (CTL) therapies, their potential in solid tumors falls short of the remarkable success achieved in blood cancers. Solid tumors present unique challenges, primarily due to their hindered accessibility, which renders adoptively transferred CTLs hypofunctional before they can reach their targets [1]. Strikingly, recent studies reveal a rapid decline in CTL effector function within hours of adoptive transfer, even in CTLs primed with robust activation and proliferation capacity. Thus, strategies to sustain CTL functionality post-transfer are of great interest to improve solid tumor therapies [2]. Here, we present a purely biomaterial approach using Polymeric-Micropatches for CD45-Phosphatase Exclusion (PMPEs) that co-migrate with CTLs and locally reinforce CTL functionality post-transfer. Fabricated from polylactic-co-glycolide (PLGA), PMPEs are stable for over two weeks at 37°C. These PMPEs bind robustly to CTLs, withstand physiologically relevant disturbances and freeze-thaw processing, and induce micron-scale exclusion of CD45-phosphatase at the CTL contact site. This exclusion, validated through mathematical modeling and experimental evidence, is an effective means of CTL stimulation simply through binding. Furthermore, the PMPE modification drives transcriptomic changes that boost CTL effector potential. Adoptively transferred PMPE-modified CTLs show superior persistence, potent type 1 immunostimulatory responses, and excellent tolerability. They significantly improve tumor control in aggressive solid tumor models, with 50% of B16F10 melanoma-bearing mice surviving beyond 25 days, compared to none in the CTL alone group. When combined with IL15sa, a systemic therapy that amplifies CTL proliferation, 46% of mice with aggressive B16F10 melanoma survive beyond 35 days, with 15.4% achieving complete remission compared to none with the IL15sa and CTL combination alone. Altogether, with their simplicity, effectiveness, and clinical translation potential, PMPEs offer new opportunities for seamless integration into clinical CTL manufacturing, advancing solid tumor management.

Hypothesis

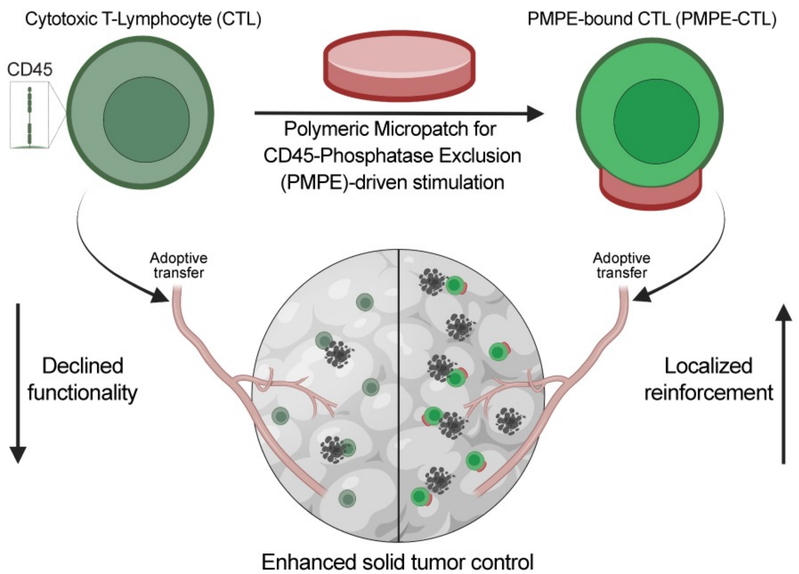

Fig. 1 Schematic illustration showing the conceptual principle of PMPE-bound CTLs (PMPE-CTLs) for post-ACT localized reinforcement to enhance solid tumor therapy. PMPE binding excludes CD45, a ubiquitously expressed surface protein on CTLs with intracellular phosphatase activity, from the binding interface. This exclusion delivers stimulation to CTLs, facilitating post-ACT reinforcement. This results in robust effector potency *in vivo* post-ACT, leading to improved control of solid tumors [3].

Results

1) Micron-scale CD45-excluding contact can stimulate CTL at even partial exclusion.

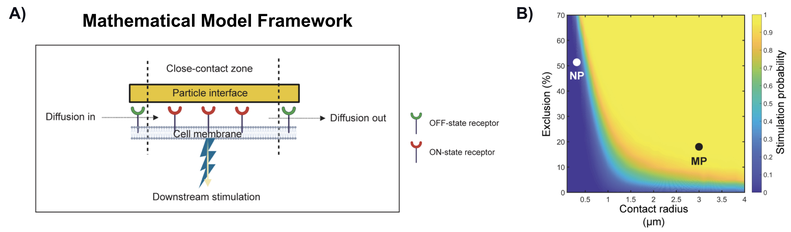

Fig. 2 (A) Representation of a mathematical model framework for CD45-exclusion-based CTL stimulation at close-contact interfaces. The mathematical model quantitatively determines CTL stimulation as a function of CD45 exclusion within close-contact zones. **(B)** Stimulation probability map indicates that micron-scale contact, such as given by micropatches (MP), achieves effective stimulation at partial exclusion (black circle), whereas nanoscale contacts, such as from nanoparticles (NP), struggle to achieve effective stimulation even at high CD45 exclusion levels (white circle).

2) PLGA-based PMPEs achieve high-efficiency CTL binding following antibody conjugation.

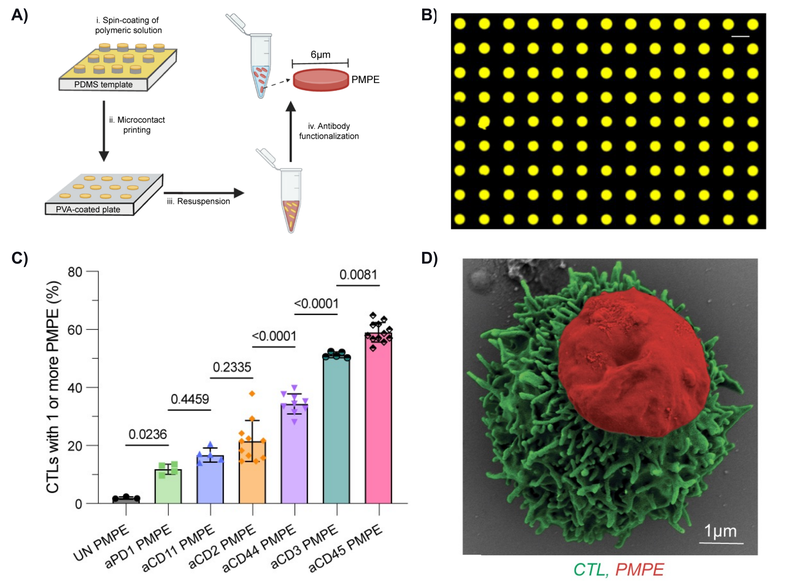

Fig. 3 (A) PMPEs are fabricated on the patterned PDMS templates. The PDMS templates are first spin-coated with the PLGA polymer solution, followed by microcontact printing on PVA-coated dishes. The printed micropatches are then functionalized with antibodies. **(B)** Fluorescent image showing PMPEs printed onto PVA-coated dishes from the spin-coated PDMS templates. Scale bar: 10 μm. **(C)** Quantification of PMPE binding to murine CTLs using flow cytometry analysis. aCD45 and aCD3 antibody-functionalized PMPEs demonstrate significantly higher binding efficiency compared to unfunctionalized (UN), aPD1-, aCD11-, aCD2-, and aCD44-functionalized PMPEs. **(D)** Pseudo-colored scanning electron micrograph of an aCD45 PMPE-CTL.

3) PMPE binding excludes CD45 receptors from the CTL contact interface.

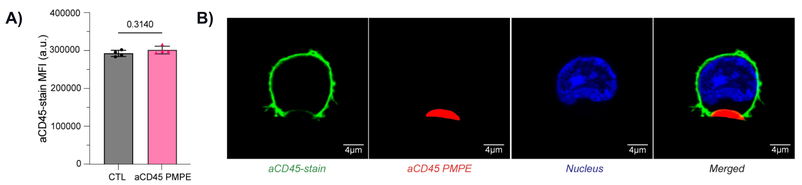

Fig. 4 Staining of CD45 receptors of CTLs was performed with or without PMPE binding. **(A)** PMPE binding does not interfere with CD45 staining of CTLs. **(B)** PMPE-bound CTLs show reduced CD45 density at the contact interface, indicating a CD45-exclusion zone. (MFI: Median fluorescence intensity, a.u.: Arbitrary unit)

4) PMPE modification stimulates CTLs without compromising their migration capability.

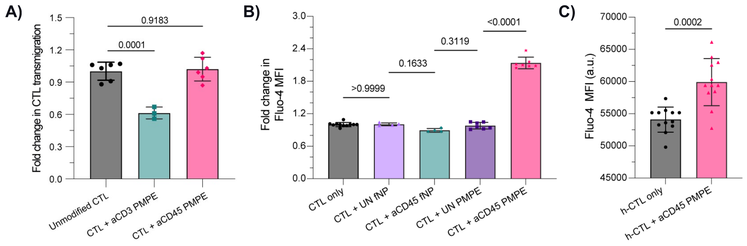

Fig. 5 (A) Effect of aCD3 or aCD45 PMPE binding on the transmigration of CTLs. aCD3 PMPE significantly reduced the CTL transmigration, while aCD45 PMPE-CTL showed similar transmigration levels as unmodified CTLs. **(B)** Fold change in Fluo-4 MFI in CTLs after 1 hr of modification. The NP and PMPE used in this experiment were fabricated with similar PLGA formulation in both unfunctionalized and functionalized groups and treated at the same mass equivalent. The Fluo-4 intensity is normalized to the intensity of unmodified CTLs in each experiment. (C) MFI of Fluo-4 in human CTLs (h-CTLs) after 1 hr of modification with aCD45 PMPEs. (MFI: Median fluorescence intensity, a.u.: Arbitrary unit)

5) Infusion of PMPE-CTLs enhances immuno-permissive activity in melanoma tumors.

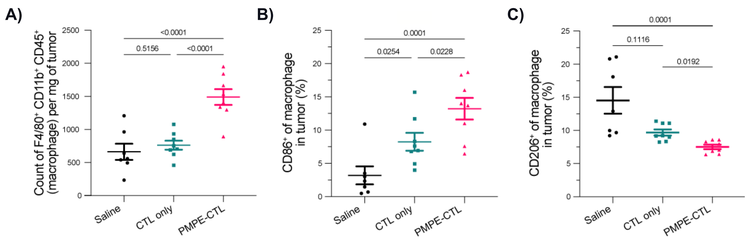

Fig. 6 Immunophenotyping analysis involved treating B16F10-melanoma-bearing mice with intravenous administration of saline, unmodified CTLs (CTL only), or PMPE-modified CTLs (PMPE-CTL) on days 7 and 10 post-tumor inoculation. Tissues were harvested for analysis 2 days following the second treatment. (A) The average count of macrophages (F4/80+CD11b+CD45+) infiltrating the tumors per mg of the tumor was significantly higher in the PMPE-CTL group compared to other groups. Tumor-infiltrating macrophages in the PMPE-CTL group exhibited a significant shift toward a pro-inflammatory M1-like phenotype (B), and a decrease in the anti-inflammatory M2-like phenotype **(C)**.

6) PMPE-CTLs outperform CTLs in treating melanoma in mice.

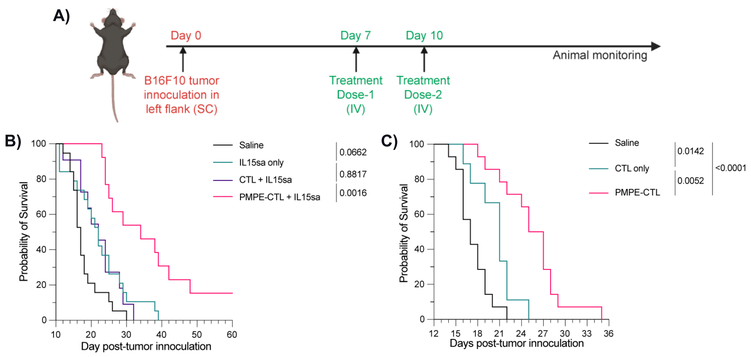

Fig. 7 (A) C57BL/6 mice were inoculated with 0.5 million B16F10 tumor cells subcutaneously and treated intravenously on days 7 and 10 with the indicated treatment. Survival curves of tumor-bearing mice as **(B)** monotherapy and **(C)** in combination with a systemic IL15-superagonist. PMPE-CTLs confer significant anti-tumor survival benefits. Pmel1-CTLs, specific for B16F10 tumor antigens, were used in all *in vivo* studies.

Reference

1. Giles J. R. et al., *Immunity*, 56 (10), 2023
2. Uslu and June, *Nature Biotechnology*, 43, 2025
3. Shaha. et al., under review