Bioengineering active immunotherapy for personalized cancer treatment

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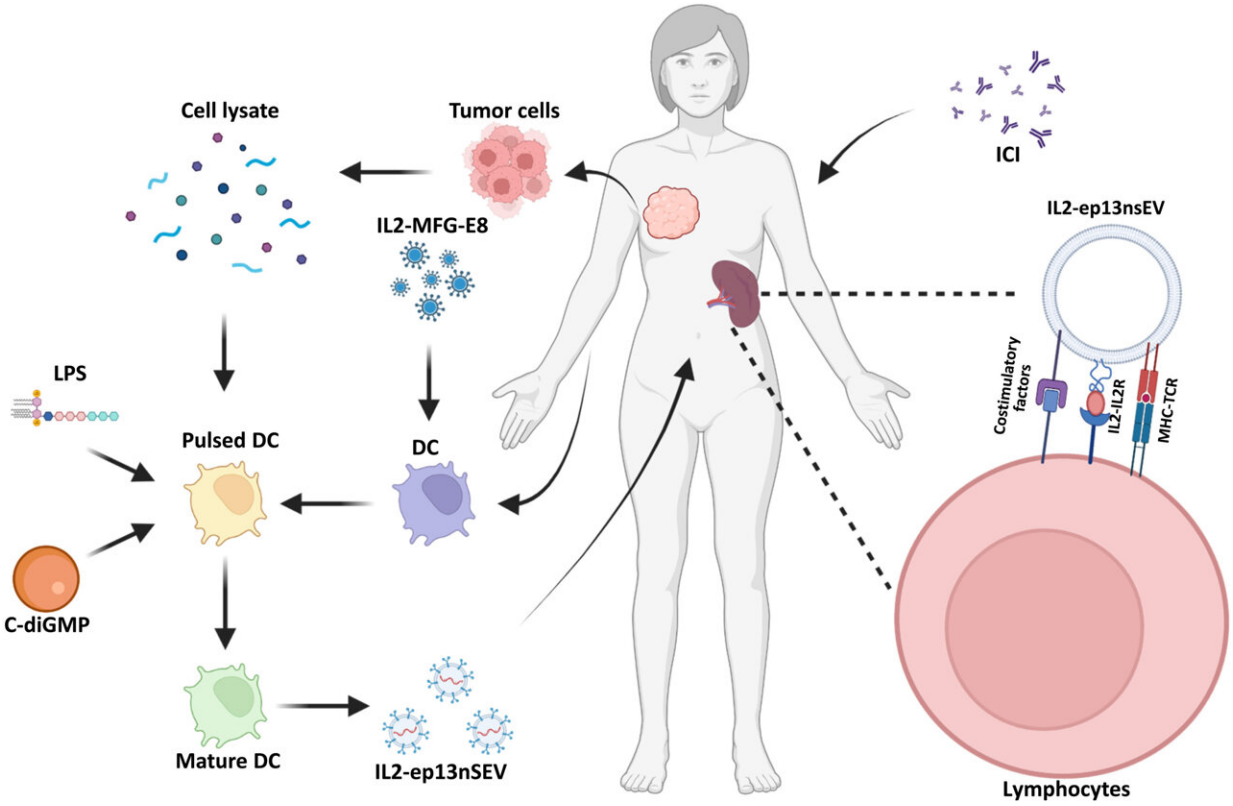


Design of IL2-ep13nsEV and its utilization in treating breast cancer. To generate this active immunotherapy, the sEVs from autologous DCs are engineered with surface membrane–bound IL2 by expressing IL2-MFG-E8. This personalization of DC-derived sEV (p13nsEV) is achieved by loading lysed surgically harvested breast cancer cells onto engineered autologous DCs followed by collecting sEVs that are then used as personalized immunotherapy. We found that LPS and STING agonist worked together to promote the expression of costimulatory factors on the surface of this engineered vesicle. Therefore, this sEV, geared

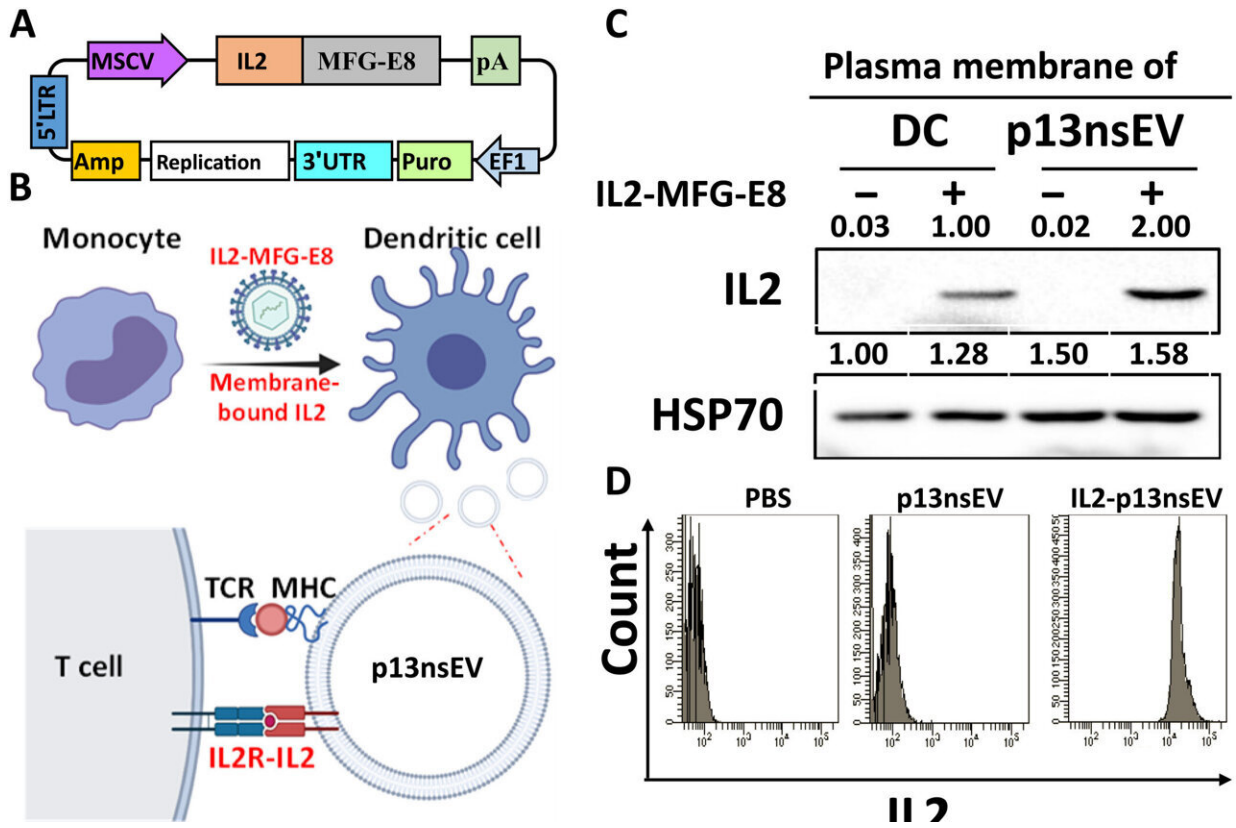
with tumor lysate–derived antigens and bioactive membrane–bound IL2 and enhanced with costimulatory factors, is named as IL2-ep13nsEV. IL2-ep13nsEV is designed to act as active immunotherapy to expand the pool of cancer-specific immune cells by facilitating neoantigen processing and presentation, as well as T cell activation. It can be used to prevent the recurrence of surgically removed primary tumor or to treat advanced breast cancer resistant to ICI. TCR, T cell receptor. Credit: *Science Advances* (2023). DOI: 10.1126/sciadv.ade0625

Breast cancer is resistant to immunotherapies; therefore, bioengineers and oncologists seek to develop a slew of therapeutic strategies to overcome this challenge. In a new report in *Science Advances*, Kerui Wu and colleagues in the departments of cancer biology, translation biology and breast surgery in the U.S., and China engineered active immunotherapy to create smart nanovesicles for personalized treatment. The research team accomplished this by anchoring membrane-bound bioactive protein interleukin-2 (IL2; made by a type of T lymphocyte) to maintain enriching and T-cell promoting co-stimulatory factors on dendritic cell-derived small extracellular vesicle surfaces.

The nanovesicles displayed major-hiscompatibility complex-bound antigens from dendritic immune cells. Upon administration, they saw how the surface-bound IL2 guided nanovesicles toward lymphoid organs associated with immune cells to activate similar immune receptors on lymphocytes. The vesicles named "IL2-ep13nsEV" induced a strong immune reaction in vivo to rescue approximately 50% of mice implanted with patient-derived xenografts while sensitizing cancer cells to immune checkpoint inhibitor treatment to prevent tumor recurrence. The outcomes present a feasible strategy to treat and prevent metastatic breast cancer in preclinical models with applications across diverse cancer types.

Nanoengineered smart vesicles

Clinical oncologists and bioengineers seek to introduce several new immunotherapeutic agents to improve the clinical outcome of specific cancers. For example, monoclonal antibody-based immune checkpoint inhibitors are markedly efficient to treat patients with lung cancer, melanoma and leukemia. Most patients are, however, resistant to immune checkpoint inhibitor therapies, where acquired resistance and relapse are common upon follow-up treatment.



Engineering of membrane-bound IL2 and induction of costimulatory factors on the surface of p13nsEV. (A) The structure of lentiviral plasmid for ectopic expression of IL2-MFG-E8 fusion protein on the membrane of p13nsEV. (B) The schematic diagram illustrating the proposed approach to anchor IL2 on the surface of p13nsEV so that it will bind to IL2 receptor on T cells. (C) The

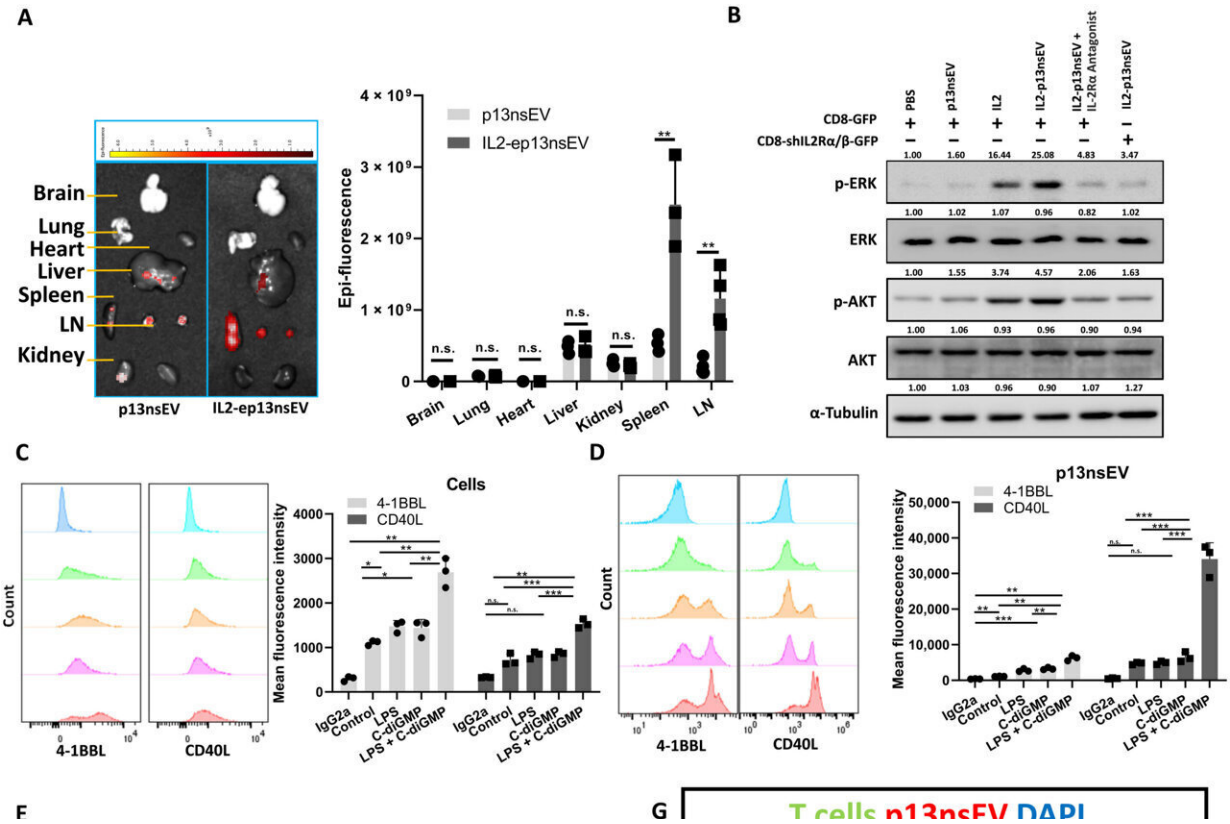
membrane fraction of p13nsEV and DCs before and after IL2 expression was isolated, and the expression of IL2 and HSP70 was examined by Western blot. (D) FC of IL2 expression on the surface of p13nsEV with or without surface IL2 expression. (E and F) Vybrant DiD–labeled T lymphocyte was cocultured with p13nsEV/PlamGFP and IL2-p13nsEV/PalmGFP. After 12 hours, the interaction between T cells and sEVs was examined and quantified by fluorescent microscopy (E) and (F) FC. N = 3 in each group. The comparison was performed using unpaired Student's t test. *P Science Advances (2023). DOI: 10.1126/sciadv.ade0625

Cancer resistance can result from low mutation burden; therefore, inhibitors are designed to enhance the anti-tumor function of T-cells, alongside a variety of active immunotherapies to generate tumor-targeted immune cells. However, the translational impact of such therapies including engineered chimeric antigen T cells can lead to life-threatening symptoms due to the controlled release of inflammatory cytokines.

Breast cancer cells are more resistant to immunotherapy due to its low mutation burden when compared to other cancer types. Wu and colleagues implemented immunotherapy through lipopolysaccharides and stimulator interferon gene agonist to promote the expression of a variety of co-stimulators on nanovesicle surfaces to generate enhanced active immunotherapy. The team loaded the vesicle with major histocompatibility complex presenting antigens derived from tumor lysates, alongside bioactive IL-2, and enriched costimulatory factors. This nanoengineered smart vesicle sought target lymphocytes and T-cells for personalized treatment for managing breast cancer.

Engineering co-stimulatory factors on the smart nanovesicle

Wu and colleagues developed active immunotherapy by engineering natural nanovesicles secreted by dendritic cells to induce specific antitumor effects via lymphocyte activation in an immune-suppressive environment. The dendritic cell-derived nanovesicles maintained essential functionality to induce immunity. The team isolated the nanovesicles from a mouse dendritic cell line and from human primary cell lines to bioengineer and name them as "p13nsEV" first, followed by "IL2-ep13nsEV" during the study. They verified the viability of the constructs and purified them, followed by electron microscopy to visualize saucer-shaped proteins expressing major hiscompatibility loci I and II on the surfaces.



IL2-ep13nsEV induces increased immunity of T cells against cancer cells. (A) The sEVs were labeled using the ExoGlow and injected into tail base of mice. After 6 hours, the organ distributions of the vesicle were examined and

quantified by IVIS spectrum. Two sailed unpaired t tests were performed to compare the signal strength in different organs. N = 4 in LN groups and N = 3 in other groups. (B) Western blot was performed for the T cells that were treated with sEVs. (C) The DCs were treated with different combinations of cytokines for stimulation. Then, 4-1BBL– and CD40L-positive DCs or p13nsEV isolated from treated DCs. (D) were examined by FC, and mean fluorescence intensity was recorded and compared by two-tailed unpaired t test. N = 3 in each group. (E) DCs with or without IL2-MFG-E8, LPS/C-diGMP enhanced costimulatory factors, were pulsed with OVA (250 μg/ml), and p13nsEV was purified. CD8 T cells from OT-I mice cells were then cocultured with different types of sEVs or pulsed DC for 5 days. FC was performed to analyze the activated IFN-γ^+ CD8 T cells. Isotype IgG1 control was used to determine the baseline signal. Two-tailed unpaired t tests were performed to compare the populations. N = 3 in each group. (F) The CD8 T cells from (E) were cocultured with B16-OVA cells expressing GFP at 10:1 ratio. FC was performed to quantify the dead cancer cells by the Zombie Aqua dead cell labeling dye among GFP^+ cells. Two-tailed unpaired t tests were performed to compare the populations. N = 3 in each group. (G) The Vybrant DiD–labeled p13nsEV was injected into mice, the LNs were taken out after 6 hours, and the sections were stained for lymphocyte markers and examined by a microscope. Scale bars, 100 μm. n.s., P ≥ 0.05, *P
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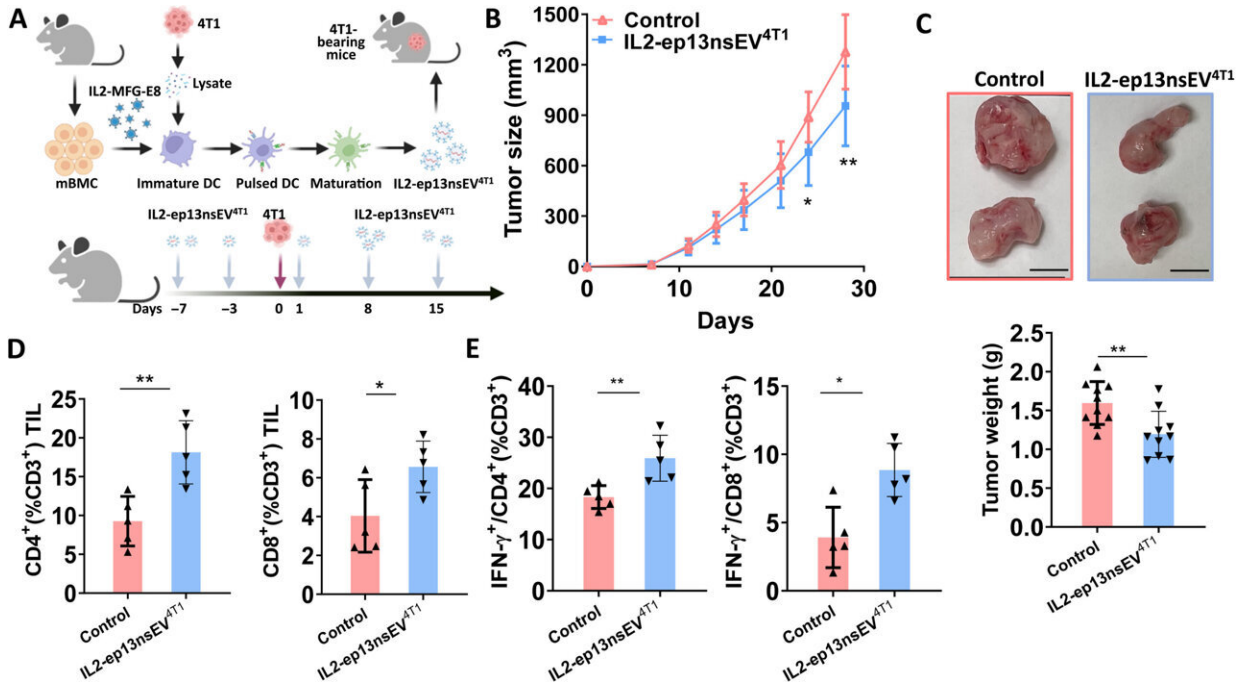
The scientists injected the nanovesicles to mice via the tail base and imaged them across various harvested organs to analyze their uptake. Wu and the team noted significant infiltration of the constructs in secondary lymphoid organs to indicate nanovesicles carrying neoantigens to immune cells for wide-spread circulation and infiltration of other immune organs.

Effects of the nanovesicles on breast tumor growth in a mouse model

The research team tested the ability of bioengineered nanovesicles to enhance cancer cell-specific killing of cytotoxic T lymphocytes in a mouse model and tracked the tumor growth of mice treated with such constructs. By four weeks they noticed the lowest tumor burden in mice treated with nanovesicles. The researchers tested if these constructs could mobilize T cells into tumor lesions and observed an increase in the natural killer cells in treated mice. They noted the significance of nanovesicle interactions with specific T cells such as CD 4 and CD 8 to suppress cancer.

The nanovesicles can work together with immune checkpoint inhibitors to enhance the effect of the latter strategy, during breast cancer treatment. While nanovesicles enable tumor growth suppression in immune-cold mouse models, it benefited the method of immune checkpoint inhibition. The two methods were complementary to rally a reactive immune response. For example, while nanovesicles generated cancer-cell specific lymphocytes, the immune checkpoint inhibitors maintained their viability to effectively eliminate cancer cells altogether. The combination of both methods increased the number of tumor-infiltrating lymphocytes and active T cells in the spleen compared to either technique alone.



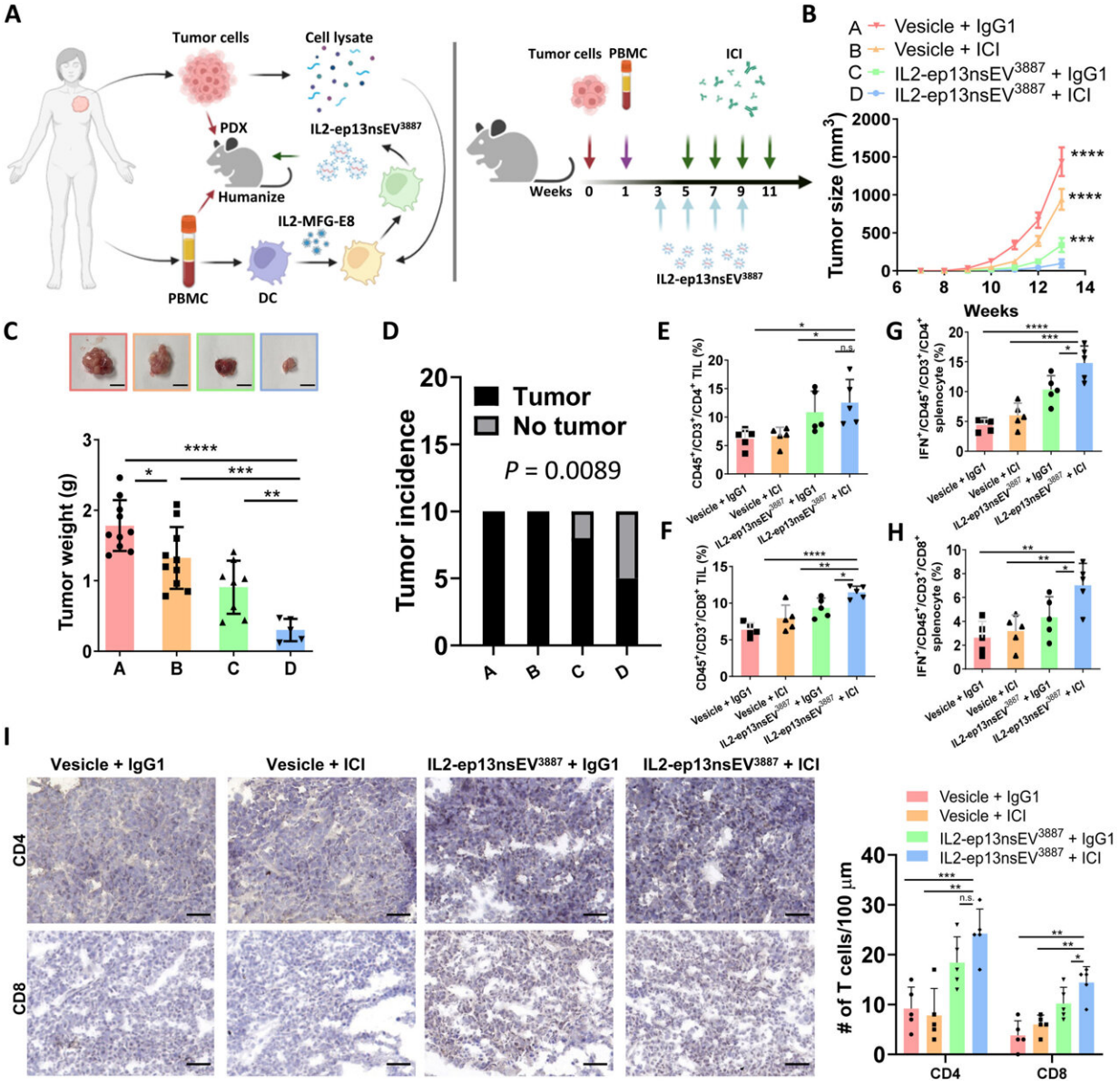
Effects of IL2-ep13nsEV on immune cold breast tumors. (A) Schematic diagram of experimental procedure. Tumor cell lysate from 4T1 was loaded into mBMDC with IL2-MFG-E8 expression, followed by DC differentiation and stimulation. The sEVs were isolated from the DC and used for the treatment of 4T1 tumor–bearing BALB/c mice. A total of 1.0×10^4 cancer cells were injected into the mammary fat pads of 6- to 8-week-old female BALB/c mice. Fifty micrograms of sEVs was given to the mice by intramuscular injection on 3 and 7 days before tumor cell implantation and 1, 8, and 15 days after tumor cell implantation. PBS was used in the control group. (B) Tumor growth on mammary fat pad was monitored by measuring the tumor size by a caliper. Two-tailed unpaired t test was performed to compare the tumor sizes. N = 10 in each group. (C) The tumor weight at the end point (day 28) is shown for the two different treatment groups. Two-tailed unpaired t test was performed to compare the tumor weight. N = 10 in each group. Scale bars, 1 cm. (D) The tumors were dissociated, $CD4^+$ and $CD8^+$ TIL among $CD3^+$ cells were measured by FC for each group, and two-tailed unpaired t test was performed to compare the percentage of TIL. N = 5 in each group. (E) The IFN-γ^+/$CD4^+$ and IFN-γ^+/$CD8^+$ among $CD3^+$ cells in dissociated splenocytes were examined by FC. The percentage of cells was compared by two-tailed unpaired t test. N = 5 in each group. Credit: *Science Advances* (2023). DOI: 10.1126/sciadv.ade0625

Preclinical investigations with a humanized patient-derived xenograft

Wu and colleagues next used a patient-derived xenograft to test the anti-tumor effect of nanoconstructs and combined the method against immune checkpoint inhibition as before. They noted the combined technologies to be more effective at tumor growth inhibition in humanized mice. The histochemistry outcomes showed how active immunotherapy vesicle treatment promoted increased T lymphocyte infiltration into the tumor.

While immune checkpoint inhibitors are administered in patients with advanced stage breast cancer, most patients undergo surgery as a curable approach at early stages. Nevertheless, patients are sometimes faced with recurrence, which leads to breast cancer–related death.

In such scenarios, Wu and colleagues recommend personalized active immunotherapy with nanovesicles to prevent future disease recurrence. They tested this theory on a mouse model and found the personalized treatment strategy could significantly decrease disease recurrence. They did not observe side-effects of the drug after 8-weeks of administration on the body weight, liver function or immune-cell hyperactivation in animals.



Effect of combination therapy in humanized PDX mice. (A) Schematic diagram of experimental procedure. Tumor cell lysate from PDX was loaded into human monocyte–derived DC with IL2-MFG-E8 expression, followed by DC differentiation and stimulation. The IL2-ep13nsEV was isolated from the DC and used as active immunotherapy to treat humanized PDX mice. Fifty micrograms of sEVs was given at weeks 3, 5, 7, and 9 after PDX implantation. The ICI treatment was given at 20 mg/kg intraperitoneally at weeks 5, 7, 9, and 11 after tumor implantation. (B) Tumor growth on mammary fat pad was monitored by measuring the tumor size by a caliper. Two-tailed unpaired t test

was performed to compare the tumor sizes at different time points. The sEVs purified from DC pulsed with lysate of health mammary fat pad were used as vesicle control of IL2-ep13nsEV^{3887}. Isotype IgG1 was used as the control of anti-PD1 treatment. N = 10 in each group. (C) The tumor weight at the end point for the four treatment groups is measured and compared by two-tailed unpaired t test. N = 10 in each group. Scale bars, 1 cm. (D) The tumor incidence of four groups was compared by the chi-square test. (E and F) The tumors were dissociated, and hCD45$^+$/hCD3$^+$, CD4$^+$(E) and CD8$^+$(F), TIL were measured by FC for each group and compared by unpaired t test. N = 5 in each group. (G and H) The hCD45$^+$/hCD3$^+$/IFN-γ$^+$, CD4$^+$ (G) and CD8$^+$ (H), cells in dissociated splenocytes were examined by FC. The percentage of cells was compared among different groups by two-tailed unpaired t test. N = 5 in each group. (I) The intratumoral CD4 and CD8 TIL were examined by IHC and compared by two-tailed unpaired t test. Scale bars, 50 μm. N = 5 in each group. n.s., P ≥ 0.05, *P
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Outlook

In this way, Kerui Wu and the research team studied breast cancer intervention in the lab; a type of cancer with highest incidence in the United States. Although non-metastatic cancers can be treated with surgery and chemotherapy, approximately 22% of patients with breast cancer eventually experience recurrence within 10 years.

Most existing therapies fall behind in saving patients with a 10-year survival rate by about 13%. As a result, the need for better therapy is imperative to treat patients at advanced stages and prevent the recurrence of disease. The outcomes of the study are promising for treating multiple cancer types alongside tumors that are resistant to the method of immune checkpoint inhibition.

More information: Kerui Wu et al, Engineering an active

immunotherapy for personalized cancer treatment and prevention of recurrence, *Science Advances* (2023). DOI: 10.1126/sciadv.ade0625

Wouter Scheper et al, Low and variable tumor reactivity of the intratumoral TCR repertoire in human cancers, *Nature Medicine* (2018). DOI: 10.1038/s41591-018-0266-5