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In vivo therapeutic cell tracking using magnetic particle imaging

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Abstract

White blood cells (WBCs) are a key component of our immune system. They play an essential role in surveillance, defense and adaptation against foreign pathogens during an immune response. Immunotherapies and immunomodulatory medications have become indispensable for treating cancer and immune disorders. Hence, imaging the immune response could help medicine diagnose and treat infections, inflammatory diseases like cardiovascular disease, and cancer. Currently, doctors rely on imaging tools like In-111 WBC scans to visualize the immune response. However, these tools destroy CAR-T and CAR-NK cells with radiation before they home to a tumor. A new biomedical imaging tool, Immuno-MPI, could remedy this pitfall and help doctors and researchers optimize immunotherapy for solid tumors. MPI uses no radiation to track cells. Its tracers also have infinite persistence. Here, we compare the effects and sensitivity limits of MPI to In111-WBC scintigraphy.

I. Introduction

The past two decades have brought impressive advancements in immune modulation, particularly with the advent of both cancer immunotherapy and biologic therapeutics for inflammatory conditions. In 2018, decades of work in immunotherapy were recognized when two immunotherapy researchers, James Allison and Tasuku Honjo, were awarded the Nobel Prize in Physiology or Medicine. Immunotherapy, the most promising new form of chemotherapy, is already the leading treatment for blood cancers, which account for 10% of all tumors. However, 90% of cancers are solid tumors, and immunotherapies have yet to revolutionize their treatments, partially due to weak efficacy and risky complications (e.g., fatal cytokine storms) [1].

Despite the emergence of the immune response's crucial role in health, the imaging field has yet to catch up to the exponential rise of immuno-oncology drugs. Presently, chimeric antigen receptor (CAR)-T and CAR-NK cells are the basis of many promising immunotherapies. Nevertheless, current radiological approaches cannot fully capture the dynamic nature of the immune response to assess therapeutic outcomes without accompanied histopathology. The standard method of white blood cell (WBC) imaging is limited to scintigraphy and single-photon emission computed tomography (SPECT), which labels the patient's autologous WBCs with an In111 tracer. The labeled WBCs are injected and then imaged hours later to highlight sites of infection, inflammation and cancer [2]. However, lymphocytes such as T and NK cells are extremely susceptible to radiation. Equivalent to 90 Gy per 10^8 cells, the radiation dose of In111 destroys the ability of lymphocytes to home to a tumor [3, 4]. Radiation also impedes their motility, induces chromosomal changes and even causes cell death [5, 6]. Hence, medicine lacks a rapid, high-throughput and quantitative *in vivo* functional imaging tool that can screen for

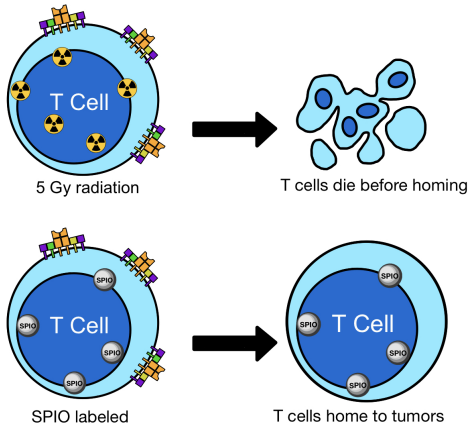

Figure 1: Experimental setup to evaluate CAR-T cell viability. CAR-T cells (lymphocytes) are exposed to 5 Gy of radiation and labeled with SPIOs respectively. In In111-WBC scintigraphy, CAR-T cells (lymphocytes) would be exposed to 90 Gy of radiation, and radiation is thought to affect their homing ability to a tumor [4, 5]. Radiation would also cause chromosomal abnormalities, induce cell death and impair migration in CAR-T cells, all of which would prevent CAR-T cells from homing to tumors. In contrast, SPIOs are thought to preserve CAR-T cell viability and functions.

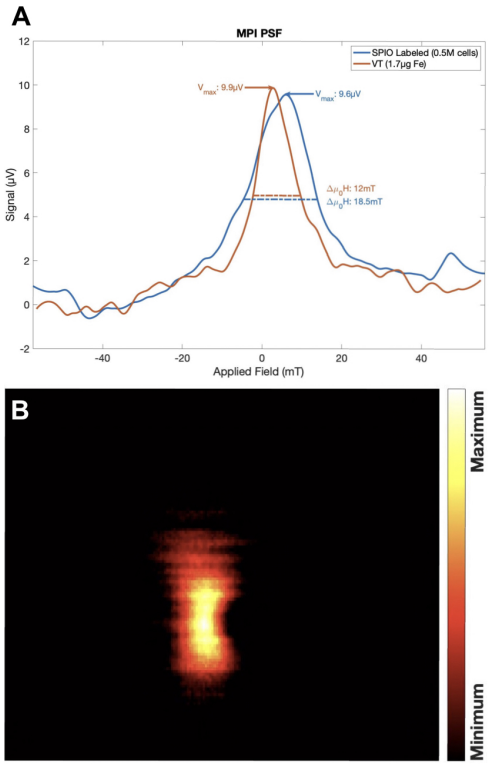

Figure 2: MPI signal of labeled CAR-T cells (A) Point spread functions (PSFs) of SPIO-labeled CAR-T cells (in blue) and SPIOs of VivoTrax (in orange). The full-width half-maximum (FWHM) resolution of the SPIO-labeled cells is 18.5 mT and 12 mT for VivoTrax. Interactions among SPIOs in the endosome of the labeled cells result in blurring, which leads to an increase in the FWHM resolution of the labeled cells. (B) MPI phantom image of labeled CAR-T cells using a 6.3 T/m gradient field-free line MPI scanner.

the most promising experimental CAR-T cell genetic manipulations to treat solid tumors.

Magnetic Particle Imaging (MPI), a new tracer-based medical imaging modality, uses non-radioactive and safe superparamagnetic iron oxide (SPIO) nanoparticle tracers that cause no damage to WBCs, including fragile CAR-T and CAR-NK cells [7, 8]. It could soon provide long-term imaging of any WBC type with zero radiation. It also has the potential to quickly and quantitatively assess novel CAR-T cell therapies' ability to target tumors with specificity. In this work, we compare MPI to nuclear medicine in terms of CAR-T cell viability and sensitivity limits.

II. Methods and materials

B3Z murine hybridoma T cell expressing receptors (TCR) that specifically recognizes ovalbumin (OVA) were cultured in culture flasks with a growth area of 75 cm^2. One flask of cells was exposed to 5 Gy of radiation, another had 100 µL of VivoTrax added to it, and the last one was used as a control. After 24 and 48 hours of incubation in culture medium, the density of cultured CAR-T cells was 165×10^4 cells/mL. The cells were then harvested and stained with annexin V-FITC and propidium iodide (PI). Fluorescence activated cell sorting (FACS) was used to analyze counts and viability. The iron concentration of labeled cells was assessed using both the UV-Vis spectrophotometer (Prussian Blue assay) and the arbitrary waveform relaxometer (AWR). VivoTrax labeled cells were imaged using a 6.3 T/m/μ_0 field-free line MPI scanner with 2D projections, a field-of-view (FOV) of 6×6 cm^2 and a 40 mTpp drive field at a frequency of 20.225 kHz. The scanning bed was mechanically translated in the z-direction in 1 mm increments to complete the imaging trajectory for a single projection.

III. Results and Discussion

CAR-T Cell Sensitivity Limits After 24 hours of incubation with SPIOs containing 5.5 mg/mL iron, the labeled CAR-T cells had an iron content of ~ 6-7 pg of Fe per cell. As shown in figure 2, this labeling efficiency yields a cell detection sensitivity of 66,000 cells, with an SNR of 1 and an average data acquisition time of 90 s, which is competitive to scintigraphy's picomolar sensitivity per 10,000 - 100,000 cells [9]. The labeling efficiency is a factor of four lower than the labeling efficiency of stem cells in our prior published papers [7]. This variation could be attributed to the fact that CAR-T cells are less phago-

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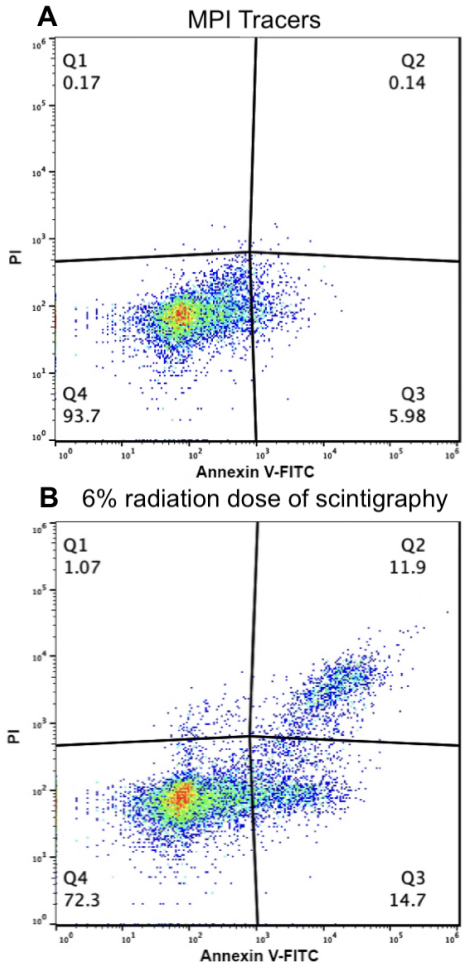

Figure 3: MPI tracers are safe and do not harm CAR-T cells, but In111-WBC scintigraphy does harm CAR-T cells. FACS analysis showed that SPIO-labeled CAR-T cells were (A) >94% alive (Q4) compared to >72% survival rate in CAR-T cells exposed to (B) 5 Gy of radiation, which is 6% of radiation dose of scintigraphy. Non-radioactive CAR-T cell tracking using MPI preserves cell viability, and thus is suitable for monitoring solid tumor therapies.

cytic compared to stem cells. Perhaps, labeling efficiency could be improved using transfection reagents such as protamine sulfate that enhances the permeability of the particles into the CAR-T cells.

CAR-T Cell Viability After 48 hours of incubation, FACS analysis showed that the early apoptotic cell population (Q3) increased from 6% for SPIO-labeled cells to 15% for 5 Gy irradiated cells. Similarly, the necrotic cell population (Q2) increased from 0.1% for SPIO-labeled cells to 12% for 5 Gy irradiated cells. This result suggests that radiation causes apoptosis in CAR-T cells while SPIOs showed no visible effect on cell viability. Hence, In111-WBC scintigraphy is not a suitable CAR-T or CAR-NK cell imaging because radiation kills WBCs far too quickly. However, MPI-WBC imaging uses zero radiation, which would scan CAR-T cells without any harm.

IV. Conclusion

CAR-T-based immunotherapies are very promising for treating tumors that have failed to respond to chemotherapy and radiation therapy. However, current imaging tools (e.g., In111-WBC scintigraphy) cannot directly track and quantify live CAR-T or CAR-NK cells without harm. Since MPI uses zero radiation SPIO tracers that cause no damage to WBCs, including fragile CAR-T and CAR-NK cells, successful demonstration of CAR-T cell tracking via MPI will be heralded as a breakthrough by the immunotherapy community. CAR-T and CAR-NK cell imaging will be very important for optimizing CAR-T and CAR-NK cell therapies. A powerful preclinical Immuno-MPI scanner could help researchers and doctors debug immunotherapy engineering challenges for solid tumors and permit optimization of immunotherapy for each patient, ala "personalized medicine."

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Author's statement

Conflict of interest: Dr. Conolly is a co-founder of a startup company that manufactures and sells preclinical MPI scanners. Part of this work was presented at the world molecular imaging congress.

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