Assessing Oximetry Response to Chimeric Antigen Receptor T-cell Therapy against Glioma with \(^{19}\)F MRI in a Murine Model

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Conflicts of interest are listed at the end of this article.

See also commentary by Bulte in this issue.

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Purpose: To assess the cell-specific, intracellular partial pressure of oxygen (Po2) dynamics of both tumor and chimeric antigen receptor (CAR) T cells in a murine immunotherapy model.

Materials and Methods: Human glioblastoma cells or human T cells were intracellularly labeled with perfluorocarbon nanoemulsion droplet sensors prior to in vivo injection in severe combined immunodeficient mice to measure Po2 in the two cell types in response to treatment. Two main sets of experiments were performed: (a) mice were injected in the flank with perfluorocarbon-labeled human glioblastoma cells and were then inoculated with either CAR T cells or untransduced T cells or were untreated 5 days after tumor inoculation; and (b) mice with unlabeled glioblastoma tumors were inoculated with perfluorocarbon-labeled CAR T cells or untransduced T cells 5 days after tumor inoculation. Longitudinal fluorine 19 (\(^{19}\)F) spin-lattice relaxation time measurements of the tumor mass were used to ascertain absolute Po2 in vivo. Results were analyzed for significance using an analysis of variance, a linear mixed-effect model, and a Pearson correlation coefficient test, as appropriate.

Results: The intracellular tumor cell Po2 temporal dynamics exhibited delayed, transient hyperoxia at 3 days after infusion of CAR T cells, commensurate with significant tumor cell killing and CAR T-cell infiltration, as observed by bioluminescence imaging and histologic findings. Conversely, no significant changes were detected in CAR or untransduced T-cell intracellular Po2 over time in tumor using these same methods. Moreover, it was observed that the total 19F tumor cell signal quenches with treatment, consistent with rapid tissue clearance of probe from apoptotic tumor cells.

Conclusion: Cell-specific Po2 measurements using perfluorocarbon probes can provide insights into effector cell function and tumor response in cellular immunotherapeutic cancer models.

Supplemental material is available for this article.

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Recent cancer therapy efforts have focused on efficient and targeted tumor cell killing and hypoxia reduction (1,2). Adoptive cell therapy has emerged as the fourth pillar of cancer therapy, offering specific eradication of hematologic cancers. Therapeutic cell engineering is now being used to target solid tumors, which are proving to be more challenging (3,4). Roadblocks include tumor-induced immunosuppression and inefficient cell trafficking as well as poor tumor penetration and persistence (4,5). Importantly, these characteristics may be predictive of therapeutic outcome. Tumor mechanisms of immunosuppression generate chronic inflammation and hypoxia in the vicinity of the tumor, which result in increased tumor angiogenesis, recurrence, and malignant progression (1,6). Effector cells in the tumor microenvironment can induce cell killing, and we hypothesize that tumor oximetry is altered as an indirect consequence of these apoptotic processes.

Recent advances in noninvasive imaging and biosensor probe technologies enable the noninvasive, real-time observation of the intracellular partial pressure of oxygen (Po2) during T-cell–mediated immunotherapy. Moreover, perfluorocarbon (PFC) exhibits weak molecular cohesion, enabling gas dissolution (7). This intrinsic property was first exploited in the 1990s (8) using emulsified PFC to form biocompatible and Injectable oxygen-laden blood substitutes and breathing liquids (9,10). Gas dissolved in fluorinated emulsions is not bound to the carrier but rather is exchanged with the local environment (11). Dissolution of oxygen in PFC lowers the fluorine 19 (\(^{19}\)F) spin-lattice relaxation time (T1) (10,12). The T1 varies linearly with the absolute Po2, which is calculated from a linear calibration curve (13–16). Thus, one can exploit the intracellular PFC label, with its intrinsic Po2 sensing properties, to perform cell-specific oximetry in vivo (15,16).
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Abbreviations

BLI = bioluminescence imaging, \(\text{CAR} = \) chimeric antigen receptor, PBS = phosphate-buffered saline, PFC = perfluorocarbon, \(\text{Po}_2 = \) partial pressure of oxygen, RARE = rapid acquisition with relaxation enhancement, R1 = relaxation rate, TAT = transactivating transcription sequence, TE = echo time, TR = repetition time, TUNEL = terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling, T1 = spin-lattice relaxation time, U87-EGFRvIII-Luc = cell line overexpressing epidermal growth factor receptor variant III and the luciferase gene

Summary

Fluorine 19 \((^{19}\text{F})\) MRI enables temporal measurements of tumor cell oxygen tension in response to chimeric antigen receptor T-cell therapy; these data support the view that \(^{19}\text{F}\) partial pressure of oxygen MRI can provide insights into the modes of action of engineered T-cell immunotherapy against cancer.

Key Points

- Longitudinal tumor cell oximetry displays a delayed, transient hypoxia upon infusion of chimeric antigen receptor T-cell therapy, thus providing insights into effector cell function in vivo.
- In contrast to tumor cell oximetry, oximetry changes in fluorine-labeled T cells were not resolved using these methods.
- The fluorine 19 \((^{19}\text{F})\) MRI signal loss of labeled tumor cells upon T-cell treatment correlated to tumor killing, as observed by bioluminescence imaging, suggests effective tissue clearance of \(^{19}\text{F}\) probe in apoptotic cells.

Our approach uses PFC nanoemulsion imaging tracer probes, used in conjunction with \(^{19}\text{F}\) MRI, for background-free cell detection (16,17). Ex vivo labeling of cells with PFC nanoemulsion can be performed by addition to the culture media. The PFC nanoemulsion is only taken up by viable cells, which retain the tag without cell efflux. Following delivery of the labeled cells to the patient, \(^{19}\text{F}\) MRI enables quantitative cell detection (ie, in vivo cytometry), a method that has been demonstrated clinically (18). Upon death of the labeled cells, PFC droplets disperse and the \(^{19}\text{F}\) signal dissipates. Rose et al (19) suggested that fluorine retention can be used as a surrogate marker for cell survival.

In this study, we test the hypothesis that a measurable change in tumor and/or T-cell \(\text{Po}_2\) is commensurate with chimeric antigen receptor (\(\text{CAR}\)) T-cell apoptotic processes in a xenograft murine model of subcutaneous glioblastoma targeted with human CAR T cells. Overall, we found that monitoring of tumor and T-cell \(\text{Po}_2\) provides an in vivo marker for monitoring cellular immunotherapeutics, and potentially for optimizing therapeutic course, dosage, and elucidation of mechanisms of action.

Materials and Methods

PFC Nanoemulsion Formulations

Aqueous nanoemulsion was gravimetrically prepared from the PFC perfluorooctyl-15-crown-5 ether (Exflur Research, Round Rock, Tex) and 5% by weight Pluronic F68 (Spectrum Chemical, Gardena, Calif) surfactant as previously described (20). Specific details of synthesis can be found in Appendix E1 (supplement).

An alternative PFC nanoemulsion formulation displayed a cell-penetrating peptide as a component of the surfactant to boost cell labeling in T cells and other weakly phagocytic cell types (21), namely, the transactivating transcription sequence (TAT) of the human immunodeficiency virus. Details on the synthesis of the TAT conjugate are found in Appendix E2 (supplement).

Nanoemulsion concentrations and \(\text{Po}_2\) calibration curve were determined by nuclear magnetic resonance. Details are described in Appendix E3 (supplement).

Human T Cells and CAR Transduction

Using anonymous donor human blood (San Diego Blood Bank, San Diego, Calif), primary human T cells were enriched by Ficoll (Histopaque-1077; Sigma Aldrich, St Louis, Mo) gradient density centrifugation and pan-T magnetic cell sorting (MACS; Miltenyi Biotech, Auburn, Calif). Protocol for CAR transduction of human T cells is found in Appendix E4 (supplement).

Glioblastoma Cells

A human glioblastoma multiform cell line (23) overexpressing the epidermal growth factor receptor variant III and the luciferase gene (U87-EGFRvIII-Luc) was maintained in T75 flasks (Sigma) in Roswell Park Memorial Institute medium, supplemented with 10% fetal bovine serum.

Ex Vivo Cell Labeling

For glioma labeling experiments, U87-EGFRvIII-Luc were plated at 90% confluence (triplicates of \(1 \times 10^6\) cells in 2 mL media per well) and incubated overnight with different PFC nanoemulsion concentrations ranging from 2.5 to 20 mg/mL. For in vivo experiments, an optimal concentration of 20 mg/mL PFC was added overnight to U87-EGFRvIII-Luc cells. Cells were counted and rinsed three times in phosphate-buffered saline (PBS), and \(5 \times 10^6\) cells were resuspended in buffered 50% Corning Matrigel (Corning Life Sciences, Tewksbury, Mass) for a 100-\(\mu\)L flank injection volume.

For intratumoral injections, CAR and untransduced T cells were labeled overnight with 15 mg/mL TAT-PFC nanoemulsion with \(10^7\) cells per well in 5 mL of media using six-well plates. Cell viability after labeling was confirmed by the trypan blue exclusion assay. The cells were washed in 0.05% trypsin for 3 minutes to remove possible extracellular label, de-beaded on a magnet, and rinsed twice in PBS. Cells were resuspended in PBS at a concentration of \(10^7\) cells per 50 \(\mu\)L for intratumoral injection. Measurement of PFC uptake after labeling is described in Appendix E3 (supplement).

Murine Model of Subcutaneous Glioblastoma

Animal protocols were approved by the University of California San Diego Institutional Animal Care and Use Committee. In the first cohort, female (\(n = 18\)) 6–8-week-old severe combined immunodeficient mice (Jackson Laboratories, Bar Harbor, Maine) received subcutaneous unilateral flank tumor injections composed of \(5 \times 10^6\) PFC-labeled glioma cells. Five
days after tumor inoculation (day 0 time point), mice were divided into three groups. Group 1 \( (n = 6) \) mice received \( 2 \times 10^7 \) CAR T cells injected intravenously in PBS. Group 2 \( (n = 6) \) mice received the same number of untransduced T cells intravenously. A second control group (group 3) remained untreated \( (n = 6) \).

In the second cohort, the same strain of mice \( (n = 12) \) received subcutaneous unilateral flank injections of \( 5 \times 10^6 \) unlabeled glioma cells. Five days after tumor inoculation (day 0 time point), mice were divided into two groups. Group 4 \( (n = 6) \) received \( 1 \times 10^7 \) TAT-PFC–labeled CAR T cells in PBS injected intratumorally, and group 5 \( (n = 6) \) received the same number of TAT-PFC–labeled untransduced T cells intratumorally.

**In Vivo Bioluminescence Imaging**

Longitudinal bioluminescence imaging (BLI) was performed on day 0, 1, 3, 7, and 10 using an IVIS Spectrum system (PerkinElmer, Waltham, Mass). d-luciferin (Intrace Medical, Lausanne, Switzerland) was administered intraperitoneally at a dose of 150 mg/kg 10 minutes prior to imaging. Mice were anesthetized (2% isoflurane in oxygen) during BLI. Regions of interest were defined as a circle encompassing the luminescent signal from each tumor, and the total flux (photons/sec) was calculated using Living Image Software (PerkinElmer). Following BLI, tumor sizes were measured using a caliper.

**MRI Scans**

MRI was performed on the same days as BLI. For groups 4 and 5, day 0 corresponds to imaging 2 hours after intratumoral injection of T cells. MRI details can be found in Appendix E5 (supplement). MRI measurements were performed with an 11.7-T Bruker BioSpec preclinical scanner (Bruker, Billerica, Mass) with a dual-tuned \( ^1H/\nu \) birdcage volume coil (Bruker).

The \( ^{19}F \) images were acquired using a rapid acquisition with relaxation enhancement (RARE) sequence with the following parameters: repetition time (TR), 2000 msec; echo time (TE), 13 msec; RARE factor, four; matrix, \( 64 \times 46 \); field of view, \( 32 \times 24 \) mm\(^2\); spatial resolution, \( 0.5 \times 0.5 \) mm\(^2\); section thickness, 1 mm; 32 averages; and 12 sections. \(^1H\) anatomic images were also acquired using the RARE sequence, with TR, 2000 msec; TE, 14 msec; RARE factor, two; matrix, \( 256 \times 184 \); field of view, \( 32 \times 24 \) mm\(^2\); spatial resolution, \( 0.125 \times 0.13 \) mm\(^2\); section thickness, 1 mm; two averages; and 12 sections. The total tumor \(^{19}F\) signal (ie, total fluorine atoms) at each time point was calculated from raw image data using Voxel Tracker software (Celsense, Pittsburgh, Pa), which incorporates the external reference and image noise as described elsewhere (24,25).

The \(^{19}F\) T1 values were measured using a point-resolved spectroscopy sequence, by defining a voxel encompassing the entire tumor mass (approximately \( 8 \times 8 \times 8 \) mm\(^3\)). Twelve TR values were used, ranging between 0.1 and 6 seconds, with a total acquisition time of approximately 30 minutes. Imaging bandwidth was adjusted to exclude potential interference of the isofluorane signals on spectra, as described elsewhere (26). The T1 values were fit using a three-parameter single exponential equation in MNova software (version 6.0.2; Mestrelab Research, Santiago de Compostela, Spain). The mean Po2 of the tumor cells and CAR T cells was calculated from T1 using a calibration curve (15,16).

**Statistical Analysis**

All measurements are presented as mean ± standard error. Results were analyzed for significance using an analysis of variance, a linear mixed-effect model, and a Pearson correlation coefficient test, as appropriate. More details can be found in Appendix E7 (supplement). Additionally, animals identical to groups 1 and 2, referred to as groups 6 and 7, were prepared \( (n = 3\), per group) for apoptotic and T-cell counts in tumors. Details on histologic sample preparation and staining for CD3 and apoptotic cells are found in Appendix E6 (supplement).

**Histologic Examination**

Additional animals identical to groups 1 and 2, referred to as groups 6 and 7, were prepared \( (n = 3\), per group) for apoptotic and T-cell counts in tumors. Details on histologic sample preparation and staining for CD3 and apoptotic cells are found in Appendix E6 (supplement).

**Results**

**Longitudinal \(^{19}F\) MRI and Glioma Burden**

To label glioma cells, PFC nanoemulsion was formulated with a mean droplet size of 176 nm ± 4 (standard deviation) and polydispersity index of 0.10 ± 0.02 as measured by dynamic light scattering. Prior to implantation, U87-EGFR-vIII-Luc cells were labeled overnight ex vivo with PFC to an average level of approximately \( 7 \times 10^{12} \) \(^{19}F\) atoms per cell, as measured with \(^{19}F\) nuclear magnetic resonance (Fig E1 [supplement]). Five days after flank tumor implantation, baseline imaging (day 0) was performed prior to cell therapy infusion. Anatomic \(^1H\) axial images show the presence of a solid tumor in the right flank of mice (Fig 1, A, left). The \(^{19}F\) image reveals a bright hotspot in the tumor in the displayed field of view (Fig 1, A, right). The \(^{19}F\) signal was detected in one to three contiguous tumor sections, where only a single section is displayed (Fig 1, A, right). Maximum pixel signal-to-noise ratio at day 0 was approximately 51. The longitudinal composite \(^1H/\nu^{19}F\) images (day 0, 7, and 10) show clearance of PFC signal over time in CAR T-cell–treated tumors (Fig 1, B). In untransduced T-cell–treated tumors, PFC hotspot remains stable at day 10 (Fig 1, C). We note that postprocessing thresholding of the pseudocolor images (Fig 1) masks low-level \(^{19}F\) signal in image display.

Longitudinal bioluminescence measurements show significant tumor burden reduction 7 days after CAR T-cell treatment with an average radiance of \( 4 \times 10^{10} \) photons/sec, which is half of the radiance measured for both naive T-cell–treated and –untreated groups (\( P = 0.007 \)) (Fig 2, A). The \(^{19}F\) content in the two control groups did not change significantly over 10 days (\( P = 0.1 \) and \( P = 0.2 \), respectively), whereas the CAR T-cell–treated tumors exhibited significant \(^{19}F\) reduction (approximately 60% signal loss; \( P = 0.001 \)) (Fig 2, B). Signal loss between groups was significant as early as day 3 (\( P = 0.03 \)) (Fig 2, B). There was a strong negative correlation between absolute tumor \(^{19}F\) content and BLI radiance in all groups (Pearson \( r = -0.85 < r < -0.98 \)) (Fig 2, C). The PFC droplets are neither broken down, nor do they
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localized 19F MR spectroscopy, with a single voxel encompassing the tumor, was used to measure relaxation rate (R1) values. Absolute tumor Po2 was calculated from the measured \(^{19}\text{F}\) signal using a calibration curve (Fig E2 [supplement]). A summary of the longitudinal tumor cell Po2 results is shown in
Figure 3, A. Notably, a transient spike was observed in tumor Po2 3 days after CAR T-cell infusion (R1 = 0.99 sec⁻¹ ± 0.12; Po2 = 134 mm Hg ± 25) (Fig 3), which was not observed for untransduced T cells (Po2 = 61 mm Hg ± 20) or controls (Po2 = 40 mm Hg ± 9; F[2,12] = 6.653; P = .0114 for analysis of variance of all day 3 groups) (Table E1 [supplement]). There was no significant Po2 change in the control groups at day 3 (t test; P = .35). By day 7, tumor oxygenation returned to baseline in the CAR T cell (Fig 3, A). These data suggest specific CAR T-cell homing to the tumor tissue, presumably initiating a target killing cascade that transiently alters intracellular Po2.

In further analysis, the R1 values for groups 1–3 are displayed as box plots in Figure 3, B. The linear mixed-effects statistical model demonstrates a significant difference in R1 between CAR T-cell–treated and untransduced T-cell–treated mice (treatment effect, 0.23; adjusted 95% CI: 0.01, 0.45) and untreated mice (treatment effect, 0.26; adjusted 95% CI: 0.05, 0.47) (Table E1 [supplement]) on day 3 with respect to day 0 that returns to baseline by day 7.

In Vivo MRI and MR Spectroscopy of T-cell Po2
To monitor effector cell oximetry in vivo, we labeled CAR T cells prior to intratumoral delivery to the flank glioma model. T cells labeled overnight with TAT-PFC nanoemulsion show no significant viability impairment (Fig E3A, E3B [supplement]). Additionally, phenotypic studies show no changes in CD4 and CD8 expression following TAT-PFC labeling (Figure E3C, E3D [supplement]). Longitudinal MRI 1H/19F overlay images of labeled CAR T cells and untransduced T cells are displayed in Figure 4. For mice receiving CAR or untransduced T cells (groups 4 and 5, respectively), day 0 corresponds to imaging 2 hours after T-cell injection. The inoculated cells remained in the tumor vicinity during the 10-day imaging period (Figs 4, 5, D). CAR T cells and untransduced T cells do not show significant changes in Po2 upon contact with tumor cells (Fig 5, A, B). The longitudinal Po2 measurements for these groups are displayed in Figure 5, A, and R1 values are displayed as box plots in Figure 5, B. The linear mixed-effects model shows no statistical significance between CAR T cells and untransduced T cells at either time point with respect to day 0 (treatment effect day 1, −0.17 [adjusted 95% CI: −0.42, 0.08]; day 3, −0.06 [95% CI: −0.32, 0.19]; day 7, −0.10 [95% CI: −0.36, 0.16]; day 10, 0.09 [95% CI: −0.17, 0.35]). When injected, CAR T cells remained cytotoxic toward cancer cells compared with untransduced T cells, as evidenced by lower tumor BLI signal as early as day 7 (P = .001) (Fig 5, C). Absolute fluorescent content of locally injected T cells in groups 4 and 5 did not change over 10 days (P = .79 and P = .26, respectively) (Fig 5, D), in contrast to labeled glioma cells (Fig 2, B). Importantly, persistence of intracellular fluorescent label in viable T cells has previously been established in vitro and in vivo in the same CAR T-cell–glioma treatment model used in this study using a fluorescent PFC emulsion and subsequent colocalization of the fluorescent signal in T cells by histopathology (22).

Histologic Assessment of the Tumor Environment after T-cell Treatment
Histologic analyses confirm intracellular localization of PFC in glioma cells and the presence of T cells in tumors (Fig E4 [supplement]). Green fluorescent protein–expressing U87 cells labeled with fluorescently conjugated PFC nanoemulsion (28) prior to implantation display intracellular localization of PFC droplets and green fluorescent protein (group 8), thus supporting the origins of the measured Po2 values. Overall, viable cells retain PFC label in lysosomal vesicles long term, as detailed elsewhere (27).

Immunohistochemical staining of tumors at day 3 after cell transfer confirms the presence of numerous CAR T cells in the tumor (group 6) and, to a lesser extent, untransduced T cells (group 7), as shown in Figure 6, A. Terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling staining revealed numerous apoptotic cells in the vicinity of CAR T cells, whereas few to no apoptotic cells were seen in untransduced T-cell–treated tumors (Fig 6, A). In groups 6 and 7, more than twice the number of CAR T cells infiltrated the tumor compared with untransduced T cells (P < .001) (Fig 6, B). CAR T-cell–treated tumor slices display numerous apoptotic tumor cells, whereas few apoptotic cells were found in untransduced T-cell–treated tumors (P < .001) (Fig 6, C). The number of CAR T cells present correlated with the number of apoptotic cells per field (Pearson r = 0.67) whereas untransduced T cells did not (Pearson r = −0.31) (Fig 6, D). These observations are consistent with CAR T cells exhibiting specific cytotoxic activity toward glioma cells in vivo. Untransduced T cells, despite reaching the tumor site, did not induce significant cell apoptosis.

Discussion
We investigated the use of PFC probe biosensors and 19F MRI for real-time monitoring of the intracellular Po2 response to CAR T-cell therapy against glioma. Peak glioma Po2 was observed 3 days after infusion and suggests significant CAR T-cell infiltration and targeted tumor cell killing, compared with untransduced T cells. Loss of glioma cell fluorine signal in the tumor correlated to reduction in tumor bioluminescence signal, suggesting effective tissue clearance of probe from apoptotic cells. In addition, CAR T-cell numbers in the tumor at day 3 correlated to cancer cell apoptosis, whereas untransduced T cells did not generate significant apoptosis. CAR T-cell and untransduced T-cell Po2 did not change significantly upon contact with tumor cells; nonetheless, the inoculated cells remained in the tumor vicinity during the imaging period (10 days). The observation of a transient increase in Po2 in glioma target with therapeutic T-cell infusion can be viewed as a real-time assay of antitumor effect and can potentially be used to evaluate cell therapy candidates preclinically, as well as to predict the optimal timing for redosing to maximize therapeutic efficacy. Moreover, Po2 increase in the CAR T-cell–treated group was short-lived, suggesting insufficient therapeutic cell homing to the tumor and possible CAR T-cell exhaustion or tumor immunosuppression (29), each being a key bottleneck in adoptive cell therapy.
Neither CAR T cells nor control T cells exhibited significant Po$_2$ changes over the course of 10 days by these techniques in this model. Generally, T cells undergo metabolic activation (30) upon binding to their specific antigen, resulting in increased oxygen consumption rate with the initiation of the killing cascade (31). We speculate that CAR T cells may tightly regulate their intracellular oxygen content, resulting in no apparent Po$_2$ change despite increased oxygen consumption rate. In addition, CAR T cells in the tumor are likely to be heterogeneous metabolically, with some cells clearly displaying effector functions, as evidenced by active tumor cell killing, and some T cells being exhausted or of CD4$^+$ phenotype. Nonetheless, CAR T cells injected intratumorally generated significant tumor growth reduction compared with their untransduced T-cell counterparts. As expected from the high number of cells delivered to the tumor, intratumoral injection of CAR T cells showed evident advantage over systemic injection based on BLI measurements ($P = .04$ at day 10). CAR T cells were not injected systemically.

**Figure 4:** Longitudinal in vivo imaging of perfluorocarbon-labeled chimeric antigen receptor (CAR) T cells delivered intratumorally. Shown are time-series proton and fluorine 19 images of the mouse abdomen bearing a flank tumor (*) with, A, perfluorocarbon-labeled CAR T cells (CAR T) or, B, untransduced T cells (untransduced T) at day (D) 0, 3, 7, and 10 after intratumoral injection. The data demonstrate accurate intratumoral delivery of T cells (hotspots) and persistent signal consistent with T-cell survival.
in the present study because homing efficiency to tumor is low in this model (19) and does not result in sufficient signal to yield reliable T1 measurements.

PFC labeling of tumor or T cells prior to injection enables uniform label distribution and circumvents biases resulting from local or systemic injection of tracer agent. Labeled cells retain the PFC label in situ, and mitosis results in symmetric partitioning of the PFC label to daughter cells (27). Po2 sensing by 19F MR spectroscopy is independent of the concentration of PFC in the cell (16); thus, cell division and probe dilution is not predicted to affect R1 measurement. Labeled cell death results in PFC dispersion and uptake predominately to Kupffer cells of the liver, as discussed elsewhere (22).

Although the mechanism of oxygen regulation in cancer cells after treatment is understudied, we speculate that the perforins released by CAR T cells upon binding to their cancer target is responsible for increased oxygen tension in the apoptotic cancer cell (32). The apoptotic pathway initiated by CAR T cells is expected to lead to tumor cell mitochondrial membrane permeabilization and reduced oxidative phosphorylation (33,34) and, potentially, reduced oxygen consumption. The Po2 measurements presented here build on prior results using 19F MR oximetry to monitor response to chemotherapy (15) and cytotoxic T cells (16). With similar methods used here, Kadayakkara et al (15) reported basal Po2 of approximately 45 mm Hg in rat glioma cells, and treatment with a chemotherapeutic agent resulted in a sustained Po2 increase to approximately 165 mm Hg over 72 hours. Zhong et al (16) used a murine model of glioma treated with Pmel-1 cytotoxic T cells and showed a transient Po2 increase (approximately 94 mm Hg) 2 days after infusion. In earlier studies, PFC nanoemulsion was directly injected intravenously, thereby enabling vasculature imaging (35–37) and tissue oximetry measurements in vivo (38–41).

Established methods for in vivo oxygenation measurements, such as electrodes or fiber-optic sensors (42,43), require invasive insertion of probes in tumors, resulting in tissue and vascular damage, inflammation, and probe sampling bias. Blood oxygen level changes can be indirectly observed by monitoring relative levels of diamagnetic oxyhemoglobin and paramagnetic deoxyhemoglobin via conventional 1H MRI (44). Hemoglobin, devoid of oxygen, shortens the relaxation times of surrounding water protons, particularly T2, giving rise to blood oxygen level–dependent MRI contrast. Blood oxygen level–dependent MRI signal changes reflect vasculature and nearby parenchyma

Figure 5: In vivo chimeric antigen receptor (CAR) T-cell partial pressure of oxygen (Po2) in tumor microenvironment. A, Summary of longitudinal T-cell Po2 measurements for intratumoral perfluorocarbon-labeled CAR T cells and untransduced T cells. B, Corresponding relaxation rate (R1) data as box plots are shown. CAR T-cell and untransduced T-cell Po2 did not vary significantly over a 10-day monitoring period. C, Bioluminescence measurements show significantly lower radiance in CAR T-cell–treated animals compared with controls at day 7 (*P < .001), indicating a therapeutic effect. By day 10, the radiance gap widens, representing significant tumor growth reduction in the CAR T-cell–treated group (*P = .009). D, Absolute fluorine content of locally injected CAR T cells and control cells, by 19F MR spectroscopy, did not change significantly over 10 days (P = .79 and P = .26, respectively), suggesting labeled T-cell persistence.
to preclinical models in its current form, but Po2 monitoring is ultimately limited by the obtainable 19F signal-to-noise ratio of the labeled cells; thus, measurement reliability may suffer at lower clinical magnetic field strengths compared with the high field strength (11.7 T) used in the present study.

Overall, we show that 19F MRI enables temporal measurements of tumor cell oxygen tension in response to CAR T-cell therapy. These data support the view that 19F Po2 MRI can provide insights into the modes of action of engineered T-cell immunotherapy against cancer. Ongoing efforts aim to understand the cellular basis of the delayed, transient hyperoxia measured in tumor cells with adoptive cell transfer, an observation that deviates from the sustained hyperoxic state seen with conventional chemotherapy delivery (15); these insights may help to improve the design of future engineered T cells and to inform the course of administered immunotherapy.

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