

Anti-tumoral effects of novel lymphatic delivery of anti-CTLA4 in a metastatic murine breast cancer model

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ABSTRACT

Cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) is an immune checkpoint receptor expressed by the activated T-cells and plays a key role in modulating T-cell function within tumor draining lymph nodes (LNs). In this study, we used a novel, infusion device, SOFUSATM, consisting of a 66 mm² microneedle array with a 6 micron polyether ketone nanotopographical film heat-formed over each microneedle to infuse therapeutic doses into the lymphatics draining LNs in mice bearing orthotopic 4T1-luc implants. Anti-CTLA-4 antibody was administered on days 11, 15, 19, and 23 via SOFUSATM. SOFUSATM infusion occurred on the right dorsal back and drained to inguinal and axillary LNs. Compared to animals dosed systemically, i.p., greater tumor shrinkage occurred and amelioration of lung metastasis occurred with SOFUSATM administration. Regional lymphatic delivery could revolutionize immune checkpoint therapy blockade immunotherapies by reducing systemic exposure, potentially alleviating immune related adverse events (irAes), and moving from systemic treatment of advanced disease, to “first-line” locoregional cancer treatment.

INTRODUCTION

Despite important clinical benefits, systemic administration of immune checkpoint blocking antibodies often result in off-target toxicities (i.e., severe immune-related adverse events, irAes) [1] possibly by inappropriately activating naive T-cells in distant LNs and other lymphoid tissues where non-tumor, self-antigens rather than tumor antigens are presented (Fig 1).

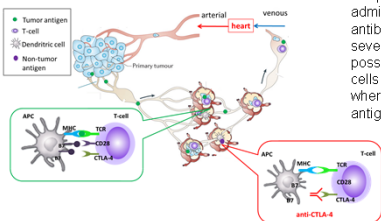


Fig 1: Sites of drug targets for locally advanced metastatic cancer. (A) In tumor-draining LNs, T-cell activation to antigen presenting cells (APC) presenting tumor antigen (Ag) can be inhibited by cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4). The tumor draining LN is a site for anti-CTLA-4 drug that blocks checkpoint inhibition by CTLA-4. (B) In regional or distant LNs where tumor-Ag may not be presented, systemic autoimmune responses can occur as an irAe to systemic CTLA-4 therapy. Reproduced in part from [2].

Efficient direct delivery of checkpoint inhibitors into the regional lymphatics could provide maximal drug exposure to targets within tumor-draining LN basins and their efferent lymphatic vessels before emptying into the blood vasculature for systemic delivery to the tumor (Fig 2). Lymphatic delivery allows regional modification of the “immune synapse” with systemic (abscopal) anti-tumor immunity.

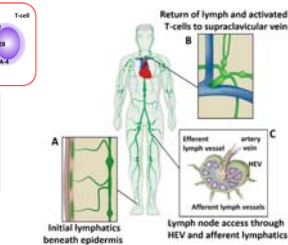


Fig 2: The open lymphatic vasculature begins at (A) the initial lymphatics located beneath the epidermis or lining of all major organs. Lymph is carried through lymphatic vessel and regional LN basins before being emptied (B) into the venous blood vasculature at the supraclavicular vein. (C) Therapeutic monoclonal antibodies can reach naive T-cells through afferent lymphatic vessels following intradermal (i.d.) administration. Adapted from [3].

METHODS

In this study, we used a novel, infusion device, SOFUSATM, consisting of a 66 mm² microneedle array with a 6 micron polyether ketone nanotopographical film heat-formed over each microneedle [4] to infuse therapeutic doses into the lymphatics in mice (Fig. 3). 4T1 mouse mammary tumor cells transfected with luciferase (4T1-luc) were injected into the mammary fat pad of BALB/C mice. CTLA-4 antibody (10mg/kg, clone 9H10) was administered on days 11, 15, 19, and 23 via SOFUSATM. SOFUSATM infusion occurred on the right dorsal back.

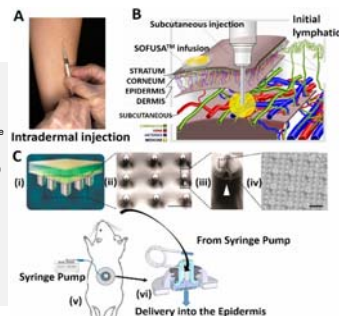


Fig 3: (A) Example of Mantoux i.d. injection (from https://en.wikipedia.org/wiki/Mantoux_test) where injection efficiently places drug at the initial lymphatics. (B) Examples of subcutaneous injection which places drug beneath the initial lymphatics, and SOFUSATM infusion with 100 microneedles that mimics i.d. delivery placement. (C) Schematic of the SOFUSATM Nanotopographical Device (i) The Microfluidic Fluid Block with a perforated attachment adhesive (tan), microfluidic distributor (green), perforated attachment adhesive (yellow), and silicon microneedle array (gray). Each microneedle is 350 µm long, 110 µm wide with a 30 µm through hole located off center which the drug flows out. (ii) Scanning Electron Microscopy (SEM) image of nanotopographical film heat formed over the silicon microneedles (scale bar represents 300 µm) (iii) SEM of individual microneedles, and (iv) SEM image of the nanostructures on each microneedle (scale bar = 3 µm). (v) Location of SOFUSATM on rat dorsal region during drug delivery experiments. (vi) Cross-sectional illustration of complete SOFUSATM device, and (ii) Microfluidic Fluid Block defined in Figure 3(Ci). Reproduced in parts from [5].

Prior work using near-infrared fluorescence (NIRF) imaging shows the device delivers directly into draining LNs (Fig 4A) and radiolabeled antibodies delivered via SOFUSATM show prolonged, but reduced PK serum profiles (Fig 5A) and enhanced LN uptake (Fig 5B) compared to i.v. administration. However, because the microarray size is developed for human use, SOFUSATM, we found accurate amounts of drug can be delivered in animals as small as rats, but radio-isotope balances must be performed to quantify dose delivered in the small (20g) mice typically used in preclinical cancer studies. Herein, we estimated that SOFUSATM delivered only 54 ± 21% of the intended, therapeutic dose of anti-CTLA-4.

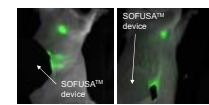


Fig 4: NIRF imaging of indocyanine green (ICG) delivery via SOFUSATM to lymphatic vessels and draining LNs on lateral side of rat. Movies of lymphatic pumping (see in reference [5]) which is a unique feature seen in rodents and humans [6] provide additional proof that therapeutic volumes of drug are being delivered to regional LN basin.

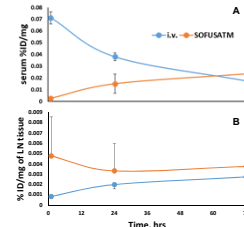


Fig 5A: Example PK serum profile (expressed as % injected dose (ID) per mg of serum) of a radiolabeled antibody administered i.v. and with SOFUSATM. The antibody employed targets a receptor target expressed on immune cells in LNs. Serum PK profiles may differ based upon antigen binding affinity and Fc characteristics.

Fig 5B: Example profile of LN uptake of radiolabeled antibody (expressed as % ID per mg of LN tissue) being delivered to regional LN basin following i.v. and SOFUSATM delivered antibody as in Fig 5A. Results confirm maximal drug exposure in LNs occur via SOFUSATM administration.

RESULTS

Despite receiving less drug as compared to animals receiving drug systemically, SOFUSATM delivery of anti-CTLA-4 resulted in reduced primary tumor growth (Fig 6) and reduced metastatic tumor burden (Fig 7, 8).

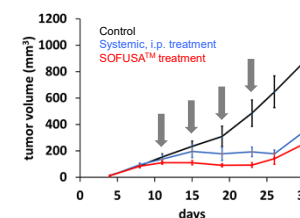


Fig 6: Growth rates of orthotopic 4T1-luc in Balb/c mice treated with control antibody (black; n=12) or with CTLA-4 (blue; n=6) administered i.p., or with SOFUSATM delivered CTLA-4 (red; n=12). Red arrows indicate treatment days. The mean tumor volumes ± SE (bars) are shown at the times that tumor measurements were made.

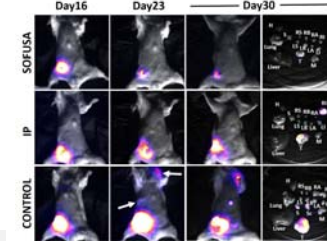


Fig 7: Bioluminescence imaging of orthotopic 4T1-luc in Balb/c mice 16, 23, and 30 days after tumor inoculation and tissues after euthanasia. CTLA-4 was treated i.p. or via SOFUSATM at 11, 15, 19, and 23 days post implantation. H, heart; RSL/L, right/left submandibular LN; RBL/L, right/left brachial LN; RALA, right/left axillary LN; RUL/L, right/left inguinal LN; T, tumor; M, muscle; S, stratum; Sc, Scapula.

Fig 8A: The lungs of mice that developed primary 4T1-luc were investigated for distant lung metastases with numbers of animals with lung mets are denoted in red and number of animals with complete response (no tumor burden in green).

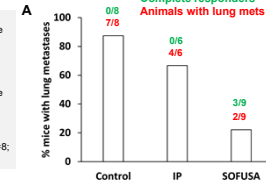
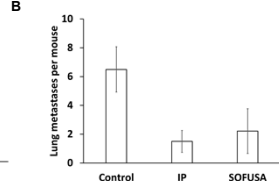


Fig 8B: Mean number of lung metastases per mouse that exhibited metastasis (control n=8; IP n=6; SOFUSATM n=9).



CONCLUSIONS

While further studies are underway, these initial imaging results suggest that lymphatic delivery of anti-CTLA-4 to tumor draining LNs could improve anti-tumor efficacy and in humans, potentially mitigate irAes associated with systemic administration which induces non-tumor antigen activation of T-cells. Furthermore, if irAes could be mitigated, then immunotherapeutics could be used at earlier stages of disease to arrest metastasis and improve cancer survival.

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REFERENCES: [1] Michot et al. Eur J Cancer 54; 139-148, 2016; [2] Stacker et al. Nat Rev Cancer 14; 159-72, 2014; [3] O'Donnell et al. J Vasc Surg Venous Lymphat Disord 5; 261-72, 2017; [4] Walsh, et al., Nanoletters, 15: 2434-41, 2015; [5] Aldrich, et al., Arthritis Research. and Therapy, 19:116, 2017; [6] Sevick-Muraca, et al., JCI, 124(3): 905-14, 2014.