

## Purity of Transferred CD8<sup>+</sup> T cells is Crucial for Safety and Efficacy of Combinatorial Tumor Immunotherapy in The Absence of SHP-1

H Angharad Watson Phd<sup>1\*</sup>, James Matthews Phd<sup>1</sup> and Ann Ager Phd<sup>1</sup>

<sup>1</sup>Division of Infection and Immunity, Systems Immunity University Research Institute, School of Medicine, Cardiff University, Henry Wellcome Building, Cardiff, CF14 4XN, UK

\*Corresponding author: H Angharad Watson, Division of Infection and Immunity, Systems Immunity University Research Institute, School of Medicine, Cardiff University, Henry Wellcome Building, Cardiff, CF14 4XN, UK, Tel: +44 (0) 29 206 87081, E-mail: [Watsonha1@cf.ac.uk](mailto:Watsonha1@cf.ac.uk)

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### Commentary

The success of immune checkpoint inhibition strategies against CTLA-4 and PD-1 in the treatment of cancer has led to the search for other inhibitory molecules that might be possible therapeutic targets. The inhibitory protein tyrosine phosphatase, SHP-1, is one such candidate molecule. This cytosolic phosphatase is widely expressed on hematopoietic cells, and negatively regulates antigen-dependent activation of T cells. In our recent study we used a unique SHP-1<sup>null</sup> mouse model to demonstrate that ablation of SHP-1 improved the ability of T cells to control both solid and diffuse tumors. In addition, we found that the absence of SHP-1 in limited numbers of certain other hematopoietic cells, including CD11b<sup>+</sup> CD11c<sup>+</sup> dendritic cells and Ly6C<sup>+</sup> macrophages was associated both with improved tumor control, and with severe and fatal lung pathology.

**Keywords:** Tumor immunotherapy, Checkpoint inhibition, SHP-1

### Introduction

As an increasing variety of tumor immunotherapy approaches are successfully translated into the clinic, the search continues for ever more ingenious manipulations of the immune system in order to improve treatment outcomes. To date, immunotherapy approaches can be broadly classified into two types; antibody-mediated checkpoint inhibition, in which the patient's immune system is manipulated on a global level to alleviate tumor-induced immune suppression [1,2]; and adoptive cell transfer, where either the patient's own tumor-specific T cells are extracted, expanded and/or modified ex-vivo before being reinfused [3,4], or chimeric antigen receptor T cells (CAR-T cells) are engineered for tumor specificity and infused into patients [5,6]. As SRC-homology domain-containing protein tyrosine phosphatase 1 (SHP-1) is a cytosolic protein, it cannot be inhibited by antibodies in the same way as anti-cytotoxic T-lymphocyte associated protein 4 (CTLA-4) and programmed death receptor-1 (PD-1), which are both cell surface proteins. Instead, approaches such as those using CRISPR/Cas9 to abrogate gene expression must be used [7], making modifying SHP-1 a "bolt-on" modification such as those being used in so-called "armored CAR" T cells [8]. Therefore, therapies targeting SHP-1 span both checkpoint inhibition and adoptive cell transfer approaches [9].

SHP-1 is an appealing modification for checkpoint inhibition as SHP-1 acts as a downstream negative regulator of antigen-specific TCR-mediated T cell activation. SHP-1 is known to bind to inhibitory-receptor superfamily (IRS) containing immunoreceptor tyrosine-based inhibitory motif (ITIM)-containing leukocyte-associated immunoglobulin receptor-1 (LAIR-1) [10]. There is strong evidence for similar interactions with other modulatory proteins such as Lck [11], and Zap70 [12]. PD-1 and CTLA-4, respectively, possesses ITIMs

and cytosolic tyrosines that could be acted upon by SHP-1, however, direct interaction between SHP-1 and these checkpoint inhibitors remains to be convincingly demonstrated [13-15]. More detail on the molecular basis of SHP-1 inhibition as an anti-cancer strategy may be found in our recent review [9].

We created a unique strain of SHP-1<sup>null</sup> mouse which survived into adulthood following genetic deletion of the interleukin-1 receptor-1, which ameliorated myeloid-driven autoimmune disease associated with the SHP-1 deficient "motheaten" mouse [16-18]. Using these mice as donors of naïve T cells, we first prevented colonization of the lungs by intravenously administered B16 melanoma cells. The protection offered by SHP-1<sup>null</sup> CD8 T cells was striking; therefore, we applied the same cells to a solid subcutaneous tumor model, again using B16 melanoma. Again, SHP-1<sup>null</sup> T cells afforded significant control of tumor growth. Analysis of tumor infiltrating T cells in the solid tumors indicated that higher numbers of T cells were found in tumors treated with SHP-1<sup>null</sup> T cells compared with WT T cells, despite equivalent numbers of T cells being transferred. Taken together with our previous studies demonstrating increased T cell expansion following TCR engagement in SHP-1<sup>null</sup> cells [19,20], this suggests that increased proliferation of T cells lacking SHP-1 following identical antigen stimulation is the mechanism behind the improved control of tumor growth. Despite significant control of tumor growth, we observed an unexpected level of morbidity and mortality amongst the animals treated with SHP-1<sup>null</sup> cells. This mortality was due to severe lung fibrosis, hemorrhage and leukocyte infiltration; all features of the original motheaten mouse. Somehow, we had transplanted motheaten pathology into the tumor bearing hosts. Analysis of the transferred T cells indicated that the SHP-1<sup>null</sup> cells behaved uniquely following CD8 enrichment using commercially available magnetic sorting kits, allowing a contaminating population of CD8 negative T cells to remain even after treatment with magnetic beads designed to remove all non-CD8 hematopoietic lineages. This contaminating population was rich in mature dendritic cells, but also included other lineages, such as macrophages. Since much of the pathology of the motheaten mouse is due to myeloid, rather than lymphoid lineages, the unexpected presence of contaminating cells bearing myeloid markers such as CD11b and CD11c is therefore a potential explanation for the observation of motheaten pathology [16,21]. Additional sorting of donor cells based on positive selection of CD8<sup>+</sup> populations resulted in a pure CD8<sup>+</sup> fraction that did not cause any pathology in host animals. However, it also did not offer the same degree of therapeutic effect on solid tumors. It was apparent that the small number of contaminating cells had anti-tumor, as well as pathological effects. Since this population was largely comprised of dendritic cells, it is not unreasonable to surmise that these SHP-1<sup>null</sup> dendritic cells were activating endogenous immune cells, and thereby augmenting the anti-

tumor effects provided by SHP-1<sup>null</sup> T cells alone. Another possibility is that they act as a feeder population for the transferred SHP-1<sup>null</sup> T cells, sustaining their expansion and survival via cytokine secretion and antigen presentation. The fact that these cells escaped capture by anti-CD11b and anti-CD11c magnetic beads, while still staining positively for these markers by flow cytometry, raises further questions about the nature of these cells, and how they might differ from conventional dendritic cells and macrophages.

The implications of this work for the clinical exploitation of SHP-1 inhibition for tumor immunotherapy are complex. On the one hand, the increased proliferative capacity of SHP-1<sup>null</sup> T cells [19] makes it an attractive modification for cells destined for adoptive transfer, as cell number can be limiting in these strategies especially following genetic modification, which can result in a certain amount of cell death. On the other hand, the degree of improvement in tumor growth control over SHP-1 sufficient T cells, although significant, might have been expected to be more dramatic. However, our model utilised a high-affinity viral TCR, while tumor-specific TCRs are notoriously low affinity. SHP-1 is involved in the regulation of TCR activation thresholds [22]; therefore in a real-world low-affinity tumor specific T cell, SHP-1 inhibition may afford a greater improvement than observed in this model system. Improvement in the stability and duration of the immune synapse following SHP-1 ablation, together with a decrease in the TCR activation threshold could allow endogenous anti-tumor TCRs to be successfully utilised therapeutically. Currently, the problem of low-affinity tumor-specific TCRs is addressed with strategies such as CAR T cells, however, bypassing the classic antigen presentation pathway has potential implications for other T cell pathways, such as homing [23]. Where an appropriate target for a CAR T cell, such as CD19, is not available, and the tumor is not rich in the neoantigens that favour current checkpoint inhibition strategies [24], ablation of SHP-1 in adoptively-transferred tumor specific T cells could provide a solution for the treatment of cancers not currently amenable to immunotherapy.

Currently, several studies and trials are focusing on the use of pharmacological phosphatase inhibitors that target SHP-1 with varying levels of specificity as anti-cancer agents [25-27], however, the lung pathology observed in our study highlights some of the potential pitfalls of global SHP-1 inhibition. Our work indicates that the cells responsible for the pathology are not a homogenous population, and it is therefore possible that one population is responsible for the anti-tumor effect, and a separate population is causing the pathology. If the anti-tumor effects of the non-CD8 SHP-1<sup>null</sup> cells could be separated from the pathological effects, then SHP-1 abrogation in these cells could be a powerful, novel anti-cancer strategy.

## Conflicts of Interest

The authors disclose no potential conflicts of interest.

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## References

- Sharma P, Allison JP (2015) Immune checkpoint targeting in cancer therapy: toward combination strategies with curative potential. *Cell* 161: 205-214.
- Sharma P, Allison JP (2015) The future of immune checkpoint therapy. See comment in PubMed Commons below *Science* 348: 56-61.
- Rosenberg SA, Packard BS, Aebersold PM, Solomon D, Topalian SL, et al. (1988) Use of Tumor-Infiltrating Lymphocytes and Interleukin-2 in the Immunotherapy of Patients with Metastatic Melanoma. *N Engl J Med* 319: 1676-1680.
- Rosenberg SA, Restifo NP (2015) Adoptive cell transfer as personalized immunotherapy for human cancer. *Science* 348: 62-68.
- Gross G, Waks T, Eshhar Z (1989) Expression of immunoglobulin-T-cell receptor chimeric molecules as functional receptors with antibody-type specificity. *Proc Natl Acad Sci U S A* 86: 10024-10028.
- Maher J, Brentjens RJ, Gunset G, Rivière I, Sadelain M (2002) Human T-lymphocyte cytotoxicity and proliferation directed by a single chimeric TCRzeta /CD28 receptor. *Nat Biotechnol* 20: 70-75.
- Lloyd A, Vickery ON, Laugel B (2013) Beyond the antigen receptor: editing the genome of T-cells for cancer adoptive cellular therapies. *Frontiers in Immunology* 4.
- Yeku OO, Brentjens RJ (2016) Armored CAR T-cells: utilizing cytokines and pro-inflammatory ligands to enhance CAR T-cell anti-tumour efficacy. *Biochem Soc Trans* 44: 412-418.
- Watson HA, Wehenkel S, Matthews J, Ager A (2016) SHP-1: the next checkpoint target for cancer immunotherapy? *Biochem Soc Trans* 44: 356-362.
- Sathish JG, Johnson KG, Fuller KJ, LeRoy FG, Meygaard L, et al. (2001) Constitutive association of SHP-1 with leukocyte-associated Ig-like receptor-1 in human T cells. *J Immunol* 166: 1763-1770.
- Chiang GG, Sefton BM (2001) Specific dephosphorylation of the Lck tyrosine protein kinase at Tyr-394 by the SHP-1 protein-tyrosine phosphatase. *The J Biological Chemistry* 276: 23173-23178.
- Plas DR, Johnson R, Pingel JT, Matthews RJ, Dalton M, et al. (1996) Direct regulation of ZAP-70 by SHP-1 in T cell antigen receptor signaling. *Science* 272: 1173-1176.
- Hebeisen M, Baitsch L, Presotto D, Baumgaertner P, Romero P, et al. (2013) SHP-1 phosphatase activity counteracts increased T cell receptor affinity. *The J Clin Invest* 123: 1044-1056.
- Yokosuka T, Kobayashi W, Takamatsu M, Sakata Sogawa K, Zeng H, et al. (2010) Spatiotemporal basis of CTLA-4 costimulatory molecule-mediated negative regulation of T cell activation. *Immunity* 33: 326-339.
- Yokosuka T, Takamatsu M, Kobayashi-Imanishi W, Hashimoto-Tane A, Azuma M, et al. (2012) Programmed cell death 1 forms negative costimulatory microclusters that directly inhibit T cell receptor signaling by recruiting phosphatase SHP2. *J Exp Med* 209: 1201-1217.
- Croker BA, Lawson BR, Rutschmann S, Berger M, Eidenschenk C, et al. (2008) Inflammation and autoimmunity caused by a SHP1 mutation depend on IL-1, MyD88, and a microbial trigger. *Proc Natl Acad Sci U S A* 105: 15028-15033.
- Shultz LD (1988) Pleiotropic effects of deleterious alleles at the "motheaten" locus. *Curr Top Microbiol Immunol* 137: 216-222.
- Watson HA, Dolton G, Ohme J, Ladell K, Vigar M, et al. (2016) Purity of transferred CD8(+) T cells is crucial for safety and efficacy of combinatorial tumor immunotherapy in the absence of SHP-1. *Immunol Cell Biol* 94: 802-808.
- Sathish JG, Dolton G, LeRoy FG, Matthews RJ (2007) Loss of Src Homology Region 2 Domain-Containing Protein Tyrosine Phosphatase-1 Increases CD8+ T Cell-APC Conjugate Formation and Is Associated with Enhanced In Vivo CTL Function. *The Journal of Immunology* 178: 330-337.
- Sathish JG, Johnson KG, LeRoy FG, Fuller KJ, Hallett MB, et al. (2001) Requirement for CD28 co-stimulation is lower in SHP-1-deficient T cells. *Eur J Immunol* 31: 3649-3658.
- Croker BA, Lewis RS, Babon JJ, Mintern JD, Jenne DE, et al. (2011) Neutrophils require SHP1 to regulate IL-1beta production and prevent inflammatory skin disease. *J Immunol* 186: 1131-1139.
- Sathish JG, Johnson KG, LeRoy FG, Fuller KJ, Hallett MB, et al. (2001) Requirement for CD28 co-stimulation is lower in SHP-1-deficient T cells. *Eur J Immunol* 31: 3649-3658.

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23. Ager A, Watson HA, Wehenkel SC, Mohammed RN2 (2016) Homing to solid cancers: a vascular checkpoint in adoptive cell therapy using CAR T-cells. *Biochem Soc Trans* 44: 377-385.
  24. Van Allen EM, Miao D, Schilling B, Shukla SA, Blank C, et al. (2015) Genomic correlates of response to CTLA-4 blockade in metastatic melanoma. *Science* 350: 207-211.
  25. Kundu S, Fan K, Cao M, Lindner DJ, Zhao ZJ, et al. (2010) Novel SHP-1 inhibitors tyrosine phosphatase inhibitor-1 and analogs with preclinical anti-tumor activities as tolerated oral agents. *J Immunol* 184: 6529-6536.
  26. Naing A, Reuben JM, Camacho LH, Gao H, Lee BN, et al. (2011) Phase I Dose Escalation Study of Sodium Stibogluconate (SSG), a Protein Tyrosine Phosphatase Inhibitor, Combined with Interferon Alpha for Patients with Solid Tumors. *Journal of Cancer* 2: 81-89.
  27. Yi T, Pathak MK, Lindner DJ, Ketterer ME, Farver C, et al. (2002) Anticancer activity of sodium stibogluconate in synergy with IFNs. *J Immunol* 169: 5978-5985.