

# SHP-1: the next checkpoint target for cancer immunotherapy?

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

<sup>\*</sup>Systems Immunity University Research Institute and Division of Infection and Immunity, School of Medicine, Henry Wellcome Building, Cardiff University, Heath Park, Cardiff, CF14 4XN, U.K.

## Abstract

The immense power of the immune system is harnessed in healthy individuals by a range of negative regulatory signals and checkpoints. Manipulating these checkpoints through inhibition has resulted in striking immune-mediated clearance of otherwise untreatable tumours and metastases; unfortunately, not all patients respond to treatment with the currently available inhibitors of cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and programmed cell death protein 1 (PD-1). Combinatorial studies using both anti-CTLA-4 and anti-PD-1 demonstrate synergistic effects of targeting multiple checkpoints, paving the way for other immune checkpoints to be targeted. Src homology 2 domain-containing protein tyrosine phosphatase 1 (SHP-1) is a widely expressed inhibitory protein tyrosine phosphatase (PTP). In T-cells, it is a negative regulator of antigen-dependent activation and proliferation. It is a cytosolic protein, and therefore not amenable to antibody-mediated therapies, but its role in activation and proliferation makes it an attractive target for genetic manipulation in adoptive transfer strategies, such as chimeric antigen receptor (CAR) T-cells. This review will discuss the potential value of SHP-1 inhibition in future tumour immunotherapy.

## Introduction

Immunotherapy has ushered in a new era in cancer treatment. Both the success of immune checkpoint inhibition strategies, and the limitations, which include non-responsiveness of some patients, as well as toxicity, has led to a search for new checkpoint targets. At the same time, the rise of cell-based immunotherapy, and an improved range of techniques for genetic modification, has expanded the range of possible targets to include intracellular checkpoints such as Src homology 2 domain-containing protein tyrosine phosphatase 1 (SHP-1). In this brief review, the potential of SHP-1 in the context of current immunotherapy strategies will be discussed.

## Checkpoint inhibition as an anti-tumour strategy

Until the start of the 21st century, all cancer treatment strategies focused on targeting and directly killing cancer cells. However, greater understanding of the regulation of T-lymphocytes in the late 1980s and 1990s led to an entirely new strategy for tumour treatment; exploiting T-cell

regulatory molecules to ‘arm’ the immune system in order to clear tumours. The first of these checkpoint inhibitors to reach the clinic was an anti-cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) antibody, ipilimumab, which first demonstrated effectiveness in the treatment of melanoma in 2008 [1,2]. This was closely followed by therapies targeting programmed death receptor-1 (PD-1) [3], the ligand for which, programmed death ligand-1 (PD-L1), is widely expressed by tumour cells [4,5]. These strategies have been recently and comprehensively reviewed elsewhere [6,7], so will not be discussed in further detail here; but their importance in signalling a sea-change in cancer therapy should not be underestimated.

## Adoptive cell therapy

Although checkpoint inhibition seeks to improve the ability of endogenous T-cells to clear tumours, adoptive transfer can take one of two approaches; *ex-vivo* expansion of a patients’ own tumour-infiltrating lymphocytes (TILs) which are then infused back into the patient [8], or generation of T-cells genetically modified to target the tumour, either through introduction of tumour-specific T-cell receptors (TCRs) or chimeric antigen receptors (CARs) [9,10], which replace the antigen recognition domain of a TCR with the epitope binding moiety of an antibody [11]. The former strategy suffers from the same limitation as checkpoint inhibition; it relies upon the existence of endogenous T-cells specific for the tumour. As tumours develop from normal tissue, many of their antigens are recognized as ‘self’, and

**Key words:** adoptive cell transfer, checkpoint inhibitors, protein tyrosine phosphatase inhibition, SHP-1, tumour immunotherapy.

**Abbreviations:** CAR, chimeric antigen receptor; CTLA-4, cytotoxic T-lymphocyte-associated protein 4, CD152; LAIR-1, leucocyte-associated immunoglobulin receptor-1; Lck, lymphocyte-specific protein tyrosine kinase; PD-1, programmed cell death protein 1, CD279; PTP, protein tyrosine phosphatase; PTPN6, protein tyrosine phosphatase, non-receptor type 6; SH2, Src homology 2 domain; SHP-1, Src homology 2 domain-containing protein tyrosine phosphatase 1, PTPN6; SSG, sodium stibogluconate; TALEN, transcription activator-like effector nucleases; TCR, T-cell receptor; TIL, tumour-infiltrating lymphocyte; Zap70, zeta-chain associated protein kinase 70.

<sup>1</sup> To whom correspondence should be addressed (email [Watsonha1@cf.ac.uk](mailto:Watsonha1@cf.ac.uk)).

those that are not are generally poorly immunogenic [12]. Mutations during tumorigenesis give rise to ‘neoantigens’; novel antigens that can be targeted by the immune system [13]. Incidence of neoantigens is associated with improved response to checkpoint inhibitor therapy [14]. Unfortunately, neoantigens are not equally distributed across cancer types [15], meaning that either checkpoint inhibition or adoptive transfer of endogenous TILs is unlikely to offer clinical benefit to patients with low-neoantigen malignancies, which include most haematological malignancies. In contrast, the greatest success to date with CAR-T-cell therapy has been with chronic lymphoid leukaemia, as circulating cancer cells may be targeted by their expression of CD19 [16]. Like any other cell-based therapy, CAR-T-cells are subject to suppression by the tumour microenvironment, and also carry the additional risk of on-target, off-tumour toxicity, including normal B-cells expressing CD19. To address these limitations, researchers are examining all aspects of CAR design, from receptor affinity [17] to adding additional properties to CAR-T-cells, such as cytokine production or release of neutralizing scFvs directed against checkpoint inhibitors in so-called ‘armoured CAR-T-cells’ [18].

## Src homology 2 domain-containing protein tyrosine phosphatase-1 in T-cells

SHP-1 [protein tyrosine phosphatase, non-receptor type 6 (PTPN6)] is expressed by all mature haematopoietic lineages and at low levels, in a different isoform, by endothelial cells [19]. There is 95% homology between human and mouse SHP-1, making it amenable for study in pre-clinical mouse models [20]. SHP-1 consists of three domains; the N-terminal Src homology-2 (SH2) domain, the C-terminal SH2 domain, and the C-terminal catalytic protein tyrosine phosphatase (PTP) domain [21]. The N-terminal SH2 domain is auto-inhibitory; binding to the PTP domain until the C-terminal SH2 domain binds to a phosphopeptide ligand, allowing a conformational change and the release of autoinhibition [21]. Maximal phosphatase activity is achieved only when both SH2 domains are engaged [22]. Given this requirement, it is likely that SHP-1 interacts with proteins of the inhibitory-receptor superfamily (IRS) containing immunoreceptor tyrosine-based inhibitory motifs (ITIMs) (I/V/LxYxxL/V) within their cytoplasmic tails [23]. It has been shown that SHP-1 constitutively interacts with ITIM-containing leucocyte-associated immunoglobulin receptor-1 (LAIR-1) [24], what is less clear is whether it directly interacts with PD-1, which also contains a cytoplasmic ITIM domain [25]. Studies in human CD4 T-cells and JURKAT cells have demonstrated co-immunoprecipitation of SHP-1 and PD-1 [26,27], however, a recent study in human CD8 T-cells found that SHP-1 and PD-1 acted independently to inhibit T-cell activation; with PD-1 preferentially inhibiting T-cells with the highest affinity TCRs, while SHP-1-mediated inhibition increased incrementally as TCR affinity increased [28]. Furthermore, only SHP-2 has been demonstrated to

interact directly with PD-1 in activated T-cells [29]. CTLA-4 does not contain any ITIMs, but does have cytosolic tyrosines that could represent potential binding sites for SHP-1, however, although other PTPs have been shown to associate with these cytosolic tyrosines, there is no direct evidence for SHP-1 interaction with CTLA-4 [30]. To date, no combinatorial studies of SHP-1 inhibition together with PD-1 or CTLA-4 inhibition have been conducted, however, the studies discussed above, in particular the work by Hebeisen et al. [28], suggest that such combinations are more likely to be synergistic than redundant in their anti-tumour effects.

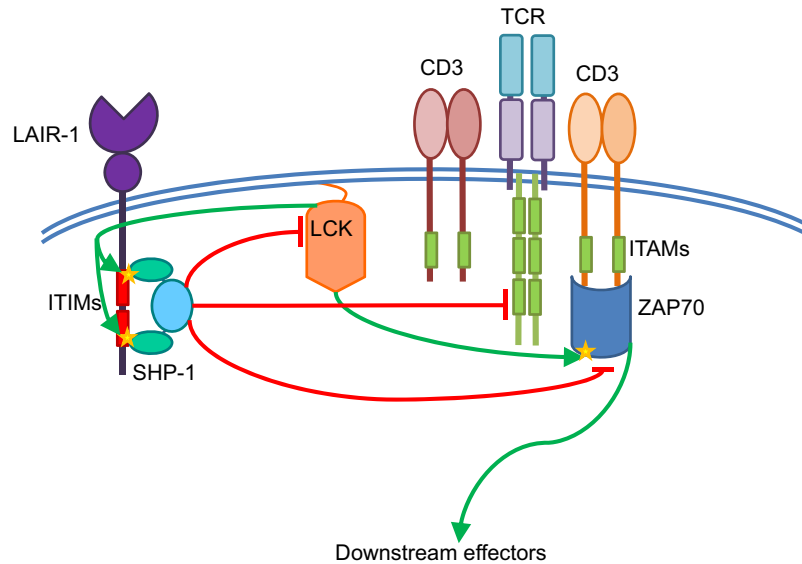
Other than LAIR-1, little is known for certain about SHP-1 binding partners in T-cells, and there is similar debate regarding its substrates, although zeta-chain associated protein kinase 70 (Zap70) [31], lymphocyte-specific protein tyrosine kinase (Lck) [32], phosphoinositide 3-kinase (PI3K) [33], Vav [34] and TCR $\zeta$  [35] are all strongly implicated [36] (Figure 1). However, the functional effect of SHP-1, or, rather, its absence, on T-cells is better understood. In the absence of SHP-1, CD8 T-cells form more stable and durable synapses with antigen presenting cells (APCs) [37]. This leads to reduced activation thresholds and increased proliferation [38], which is beneficial for any kind of adoptive transfer strategy for two reasons: firstly, numbers of T-cells available for transfer are often limited, especially where genetic modification is involved; and, secondly it is known that the balance of regulatory T-cells to effector T-cells is key in tumour progression [39], so any modification that can bias towards increased effector T-cells is likely to improve treatment efficacy (Figure 2). It is worth noting that SHP-1 has also been shown to be inhibitory to T regulatory cells [40], and therefore inhibition of SHP-1 in these cells leads to increased suppressor function. As with CD8 T-cells, this effect is attributed to increases in TCR-APC conjugate formation and duration. Specific deletion of SHP-1 in all CD4 T-cells via a floxed Shp1<sup>fl/fl</sup> CD4-cre system in mice demonstrated a key role for SHP-1 in negatively regulating the responsiveness of CD4 T-cells to interleukin-4 signalling, and therefore maintenance of a TH1 phenotype [41]. Deletion of SHP-1 in other haematopoietic lineages in mouse models, such as B-cells, neutrophils and dendritic cells, is associated with a variety of pathologies [42–45], although SHP-1<sup>-/-</sup> CD8 T-cells have not been linked to any pathological effects, to date.

## A natural model

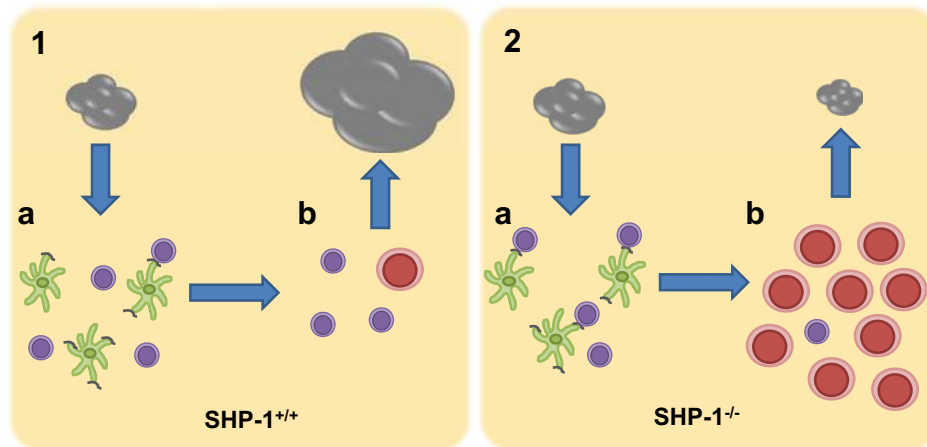
In 1965, a spontaneous recessive mutation was observed among the mice in Jackson Laboratories, and was given the name ‘motheaten’ due to the marked skin lesions observed on homozygous animals [46]. Motheaten mice die at 3–4 weeks of age, but in 1985 a new mutant mouse was described that had a mutation in the motheaten locus, but survived up to 9 weeks of age; this mouse was dubbed ‘motheaten viable’ [47]. It was not until 1993 that these mutations were associated with a haematopoietic phosphatase [48,49], later named SHP-1 by consensus. The motheaten

**Figure 1 | SHP-1 mediated inhibition of TCR signalling**

SHP-1 is constitutively associated with the inhibitory receptor LAIR-1, which, in turn, is constitutively phosphorylated by Lck [74], although SHP-1 may also be activated by other ITIM-containing inhibitory receptors. Activation of SHP-1 allows it to inhibit antigen-induced TCR signalling either through direct dephosphorylation of the TCR $\zeta$  chain, or dephosphorylation of downstream adaptor proteins such as Lck and ZAP70. Activating phosphate groups are shown as stars.

**Figure 2 | Lowered activation thresholds, increased duration of interaction with antigen presenting cells (green) and increased expansion of SHP-1<sup>-/-</sup> CD8 T-cells are beneficial in tumour therapy**

(1a) Tumour antigens (grey) are low affinity and poorly immunogenic, and offer limited stimulation to naïve T-cells (purple). (1b) Low numbers of tumour specific effector T-cells (red) are insufficient to control tumour growth. (2a) SHP-1<sup>-/-</sup> T-cells have lower activation thresholds, therefore can be stimulated by low-affinity antigens. (2b) In response to antigen stimulation SHP-1<sup>-/-</sup> T-cells proliferate more than WT T-cells, leading to increased numbers of tumour specific effector T-cells, and predicted control of tumour growth.



mouse suffers a range of pathologies, including myeloid-driven skin lesions, interstitial pneumonitis (usually fatal), and a range of haematological abnormalities; polyclonal activation of B-cells, decreased NK cell activity, haemolytic anaemia, decreased dermal dendritic cells, as well as the

previously described hyperproliferative T-cells [50,51]. The short lifespan of these animals and the range of multifactorial immunopathologies make them difficult to use effectively in the study of T-cell function. However, the extent of immune dysregulation in these animals indicates the importance of

SHP-1 in the regulation of the immune system, and further suggests that specific targeting of SHP-1 in individual cell populations might be a safer approach in patients, rather than global inhibition, as in anti-CTLA-4 and anti-PD-1 therapies.

## SHP-1 abrogation in cancer therapy

A number of strategies to exploit the benefits of SHP-1 abrogation have been attempted to date. In pre-clinical studies, adoptive transfer of SHP-1 knockout T-cells has been shown to be beneficial in a model of leukaemia [52], whereas two phase I clinical trials have been taken place to assess the safety of using systemic treatment with sodium stibogluconate (SSG), a licensed treatment for leishmaniasis that is also an active-site inhibitor of SHP-1 and the related SHP-2, as a cancer therapy [53,54]. A further pre-clinical study has looked at developing a new, orally-bioavailable (SSG must be infused intravenously) SHP-1 inhibitor; a small-molecule, aromatic compound denoted as tyrosine phosphatase inhibitor 1 (TPI-1) by the authors [55]. In this study, TPI-1 was found to be ~58 times as effective as SSG *in vitro*, and elicited an anti-tumour effect against 4-day established B16 melanomas *in vivo*, where SSG failed to have any effect. NSC-87877 is a small molecule competitive inhibitor of SHP-2, which is also inhibitory to SHP-1 [56] and is being explored as an anti-tumour agent, however this is due to its inhibitory effects on dual specificity protein phosphatase 26 (DUSP 26), which is overexpressed in neuroblastoma, rather than as a result of SHP-1 inhibition [57]. Suramin is another anti-parasitic agent that has been found to mediate active-site inhibition of a range of PTPs, and is therefore being investigated as an anti-tumour agent, however, its wide spectrum of target PTPs puts it beyond the scope of this review [58]. Historically, active-site-directed inhibitors of PTPs have been challenging due to the problem of creating cell membrane-permeable yet highly negatively charged compounds, however, recently, a cryptic allosteric inhibition site has been successfully targeted in SHP-2 [59], which represents a new strategy for PTP inhibition that might improve the clinical applicability of PTP inhibition.

In the study by Stromnes et al. [52], the authors used an Lck-driven cre to knockout floxed SHP-1 in mature T-cells. This system was used in preference to the SHP-1<sup>null</sup> motheaten mouse, in order to avoid any confounding influence of other aberrantly activated SHP-1<sup>null</sup> immune cells [60] on the maturation of the T-cells. In order to mimic clinical adoptive transfer strategies, T-cells were subject to three rounds of *in vitro* antigen stimulation prior to transfer. Although this system might appear to fail to take advantage of the increased antigen-dependent proliferation of naive SHP-1<sup>null</sup> T-cells described by Sathish et al. [37,61], the authors observed increased proliferation of transferred effector T-cells in response to tumour *in vivo*, reduced apoptosis and improved survival of SHP-1<sup>-/-</sup> T-cells, and, ultimately improved clearance of leukaemia. This demonstrates that

abrogation of SHP-1 is beneficial in effector T-cells, not just in naive T-cells, and therefore knocking out SHP-1 in *in vitro*-activated, genetically modified T-cells would still add value to adoptive transfer strategies.

To date, although carried out in cancer patients, clinical trials of small-molecule SHP-1 inhibitors remain restricted to phase I dosing studies, and therefore anti-tumour effects, although measured, were not the primary purpose of the studies. In the event, no clinically measurable anti-tumour effects were observed in either study [53,54]. Although not the purpose, this is disappointing and does bring into question the effectiveness of SSG administration as an anti-cancer strategy. No phase II studies of small-molecule SHP-1 inhibition have been completed. Evaluation of toxicity of SSG was somewhat limited in both studies due to the combination of SSG with interferon and/or chemotherapy, and therefore where severe and/or life threatening adverse effects were observed (in up to 68% of patients), it was difficult to establish which treatment was responsible. Dose-limiting toxicities observed included pancreatitis, bone marrow suppression, fatigue, lipase elevation and gastrointestinal upset. Not observed was the fatal cardiac toxicity seen in 5–7% of leishmaniasis patients treated with SSG [62]. Both studies concluded that SSG treatment was well tolerated.

Interestingly, especially when considering global SHP-1 inhibition with agents such as SSG or TPI-1, SHP-1 expression is altered in a range of malignancies; up-regulated in breast and ovarian cancers [63,64], and gene-silenced in lymphomas, leukaemias and colorectal cancers [65–67].

## Future strategies

The disappointing performance of SSG/TPI-1 as an anti-cancer agent in both the pre-clinical and clinical studies described above suggests that the adoptive transfer approach of Stromnes et al. [52] might be the most promising avenue for exploitation of SHP-1 inhibition for tumour therapy. The cytosolic nature of SHP-1, and the difficulty in identifying inhibitors that will not target SHP-2 and other PTPs, means that genetic manipulation would be the best strategy for translational studies. There are currently a range of different techniques available for genetic manipulation that have been utilized in various adoptive transfer and CAR-T-cell approaches. A recent study used zinc finger nucleases via RNA electroporation to knockout PD-1 in TILs on a clinical scale in order to treat metastatic melanoma [68], however, limited success meant only *in vitro* evaluation of the modified cells was possible. In our own lab, we are currently investigating a zinc finger nuclease approach for ablating SHP-1 in human CD8 T-cells for tumour therapy. In the past, lenti- and retrovirally mediated gene transfer strategies have been popular, but difficulties with transduction of T-cells has led to electroporation of either DNA or RNA becoming the method of choice. CAR-T-cell therapies have optimized a number of genetic modification approaches, including the Sleeping Beauty transposon system [69],

clustered regularly interspaced short palindromic repeats (CRISPR) [70] and transcription activator-like effector nucleases (TALEN) [71]. These approaches are reviewed in more detail elsewhere [72,73]. However, the range of clinically applicable gene transfer techniques available today mean that the additional knockout of a molecule like SHP-1 from T-cells already undergoing genetic modification becomes a much more straightforward proposition, making it more likely that the beneficial anti-cancer properties of SHP-1<sup>-/-</sup> T-cells can be exploited in the clinic in the near future.

## Funding

This work was supported by The Wellcome Trust [grant number 094511/Z/10/Z (to H.A.W., J.M., A.A.)]; the Medical Research Council UK [grant number MR/L008742/1 (to H.A.W., S.W., A.A.)]; and S.W. was supported by a Medical Research Council UK PhD studentship.

## References

- 1 Yuan, J., Gnjatic, S., Li, H., Powel, S., Gallardo, H.F., Ritter, E., Ku, G.Y., Jungbluth, A.A., Segal, N.H., Rasalan, T.S. et al. (2008) CTLA-4 blockade enhances polyfunctional NY-ESO-1 specific T cell responses in metastatic melanoma patients with clinical benefit. *Proc. Natl. Acad. Sci. U.S.A.* **105**, 20410–20415 [CrossRef PubMed](#)
- 2 Hodi, F.S., Butler, M., Oble, D.A., Seiden, M.V., Haluska, F.G., Kruse, A., MacRae, S., Nelson, M., Canning, C., Lowy, I. et al. (2008) Immunologic and clinical effects of antibody blockade of cytotoxic T lymphocyte-associated antigen 4 in previously vaccinated cancer patients. *Proc. Natl. Acad. Sci. U.S.A.* **105**, 3005–3010 [CrossRef PubMed](#)
- 3 Brahmer, J.R., Drake, C.G., Wollner, I., Powderly, J.D., Picus, J., Sharfman, W.H., Stankevich, E., Pons, A., Salay, T.M., McMiller, T.L. et al. (2010) Phase I study of single-agent anti-programmed death-1 (MDX-1106) in refractory solid tumors: safety, clinical activity, pharmacodynamics, and immunologic correlates. *J. Clin. Oncol.* **28**, 3167–3175 [CrossRef PubMed](#)
- 4 Droeser, R.A., Hirt, C., Viehl, C.T., Frey, D.M., Nebiker, C., Huber, X., Zlobec, I., Eppenberger-Castori, S., Tzankov, A., Rosso, R. et al. (2013) Clinical impact of programmed cell death ligand 1 expression in colorectal cancer. *Eur. J. Cancer* **49**, 2233–2242 [CrossRef PubMed](#)
- 5 Avril, T., Saikali, S., Vauleon, E., Jary, A., Hamlat, A., De Tayrac, M., Mosser, J. and Quillien, V. (2010) Distinct effects of human glioblastoma immunoregulatory molecules programmed cell death ligand-1 (PDL-1) and indoleamine 2,3-dioxygenase (IDO) on tumour-specific T cell functions. *J. Neuroimmunol.* **225**, 22–33 [CrossRef PubMed](#)
- 6 Sharma, P. and Allison, J.P. (2015) Immune checkpoint targeting in cancer therapy: toward combination strategies with curative potential. *Cell* **161**, 205–214 [CrossRef PubMed](#)
- 7 Sharma, P. and Allison, J.P. (2015) The future of immune checkpoint therapy. *Science* **348**, 56–61 [CrossRef PubMed](#)
- 8 Rosenberg, S.A., Packard, B.S., Aebbersold, P.M., Solomon, D., Topalian, S.L., Toy, S.T., Simon, P., Lotze, M.T., Yang, J.C., Seipp, C.A. 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 [CrossRef PubMed](#)
- 9 Gross, G., Waks, T. and Eshhar, Z. (1989) Expression of immunoglobulin-T-cell receptor chimeric molecules as functional receptors with antibody-type specificity. *Proc. Natl. Acad. Sci.* **86**, 10024–10028 [CrossRef](#)
- 10 Maher, J., Brentjens, R.J., Gunset, G., Riviere, I. and Sadelain, M. (2002) Human T-lymphocyte cytotoxicity and proliferation directed by a single chimeric TCR[ $\zeta$ ]/CD28 receptor. *Nat. Biotechnol.* **20**, 70–75 [CrossRef PubMed](#)
- 11 Rosenberg, S.A. and Restifo, N.P. (2015) Adoptive cell transfer as personalized immunotherapy for human cancer. *Science* **348**, 62–68 [CrossRef PubMed](#)
- 12 Aleksic, M., Liddy, N., Molloy, P.E., Pumphrey, N., Vuidepot, A., Chang, K.-M. and Jakobsen, B.K. (2012) Different affinity windows for virus and cancer-specific T-cell receptors – implications for therapeutic strategies. *Eur. J. Immunol.* **42**, 3174–3179
- 13 Gubin, M.M., Zhang, X., Schuster, H., Caron, E., Ward, J.P., Noguchi, T., Ivanova, Y., Hundal, J., Arthur, C.D., Krebber, W.-J. et al. (2014) Checkpoint blockade cancer immunotherapy targets tumour-specific mutant antigens. *Nature* **515**, 577–581 [CrossRef PubMed](#)
- 14 Van Allen, E.M., Miao, D., Schilling, B., Shukla, S.A., Blank, C., Zimmer, L., Sucker, A., Hillen, U., Geukes Foppen, M.H., Goldinger, S.M. et al. (2015) Genomic correlates of response to CTLA-4 blockade in metastatic melanoma. *Science* **350**, 207–211 [CrossRef PubMed](#)
- 15 Lawrence, M.S., Stojanov, P., Polak, P., Kryukov, G.V., Cibulskis, K., Sivachenko, A., Carter, S.L., Stewart, C., Mermel, C.H., Roberts, S.A. et al. (2013) Mutational heterogeneity in cancer and the search for new cancer-associated genes. *Nature* **499**, 214–218 [CrossRef PubMed](#)
- 16 Maude, S.L., Frey, N., Shaw, P.A., Aplenc, R., Barrett, D.M., Bunin, N.J., Chew, A., Gonzalez, V.E., Zheng, Z., Lacey, S.F. et al. (2014) Chimeric antigen receptor T cells for sustained remissions in Leukemia. *N. England J. Med.* **371**, 1507–1517 [CrossRef](#)
- 17 Caruso, H.G., Hurton, L.V., Najjar, A., Rushworth, D., Ang, S., Olivares, S., Mi, T., Switzer, K., Singh, H., Huls, H. et al. (2015) Tuning sensitivity of CAR to EGFR density limits recognition of normal tissue while maintaining potent antitumor activity. *Cancer Res.* **75**, 3505–3518 [CrossRef PubMed](#)
- 18 Pegram, H.J., Park, J.H. and Brentjens, R.J. (2014) CD28z CARs and armored CARs. *Cancer J.* **20**, 127–133 [CrossRef PubMed](#)
- 19 Lorenz, U. (2009) SHP-1 and SHP-2 in T cells: two phosphatases functioning at many levels. *Immunol. Rev.* **228**, 342–359 [CrossRef PubMed](#)
- 20 Matthews, R.J., Bowne, D.B., Flores, E. and Thomas, M.L. (1992) Characterization of hematopoietic intracellular protein tyrosine phosphatases: description of a phosphatase containing an SH2 domain and another enriched in proline-, glutamic acid-, serine-, and threonine-rich sequences. *Mol. Cell. Biol.* **12**, 2396–2405 [CrossRef PubMed](#)
- 21 Yang, J., Liu, L., He, D., Song, X., Liang, X., Zhao, Z.J. and Zhou, G.W. (2003) Crystal structure of human protein-tyrosine phosphatase SHP-1. *J. Biol. Chem.* **278**, 6516–6520 [CrossRef PubMed](#)
- 22 Pluskey, S., Wandless, T.J., Walsh, C.T. and Shoelson, S.E. (1995) Potent stimulation of SH-PTP2 phosphatase activity by simultaneous occupancy of both SH2 domains. *J. Biol. Chem.* **270**, 2897–2900 [CrossRef PubMed](#)
- 23 Long, E.O. (1999) Regulation of immune responses through inhibitory receptors. *Annu. Rev. Immunol.* **17**, 875–904 [CrossRef PubMed](#)
- 24 Sathish, J.G., Johnson, K.G., Fuller, K.J., LeRoy, F.G., Meygaard, L., Sims, M.J. and Matthews, R.J. (2001) Constitutive association of SHP-1 with leukocyte-associated Ig-like receptor-1 in human T cells. *J. Immunol.* **166**, 1763–1770 [CrossRef PubMed](#)
- 25 Carter, L., Fouser, L.A., Jussif, J., Fitz, L., Deng, B., Wood, C.R., Collins, M., Honjo, T., Freeman, G.J. and Carreno, B.M. (2002) PD-1:PD-L inhibitory pathway affects both CD4(+) and CD8(+) T cells and is overcome by IL-2. *Eur. J. Immunol.* **32**, 634–643 [CrossRef PubMed](#)
- 26 Chemnitz, J.M., Parry, R.V., Nichols, K.E., June, C.H. and Riley, J.L. (2004) SHP-1 and SHP-2 associate with immunoreceptor tyrosine-based switch motif of programmed death 1 upon primary human T cell stimulation, but only receptor ligation prevents T cell activation. *J. Immunol.* **173**, 945–954 [CrossRef PubMed](#)
- 27 Sheppard, K.-A., Fitz, L.J., Lee, J.M., Benander, C., George, J.A., Wooters, J., Qiu, Y., Jussif, J.M., Carter, L.L., Wood, C.R. and Chaudhary, D. (2004) PD-1 inhibits T-cell receptor induced phosphorylation of the ZAP70/CD3 $\zeta$  signalosome and downstream signaling to PKC $\theta$ . *FEBS Lett.* **574**, 37–41 [CrossRef PubMed](#)
- 28 Hebeisen, M., Baitsch, L., Presotto, D., Baumgaertner, P., Romero, P., Michielin, O., Speiser, D.E. and Rufer, N. (2013) SHP-1 phosphatase activity counteracts increased T cell receptor affinity. *J. Clin. Invest.* **123**, 1044–1056 [CrossRef PubMed](#)
- 29 Yokosuka, T., Takamatsu, M., Kobayashi-Imanishi, W., Hashimoto-Tane, A., Azuma, M. and Saito, T. (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 [CrossRef PubMed](#)



- 30 Yokosuka, T., Kobayashi, W., Takamatsu, M., Sakata-Sogawa, K., Zeng, H., Hashimoto-Tane, A., Yagita, H., Tokunaga, M. and Saito, T. (2010) Spatiotemporal basis of CTLA-4 costimulatory molecule-mediated negative regulation of T cell activation. *Immunity* **33**, 326–339 [CrossRef PubMed](#)
- 31 Plas, D.R., Johnson, R., Pingel, J.T., Matthews, R.J., Dalton, M., Roy, G., Chan, A.C. and Thomas, M.L. (1996) Direct regulation of ZAP-70 by SHP-1 in T cell antigen receptor signaling. *Science* **272**, 1173–1176 [CrossRef PubMed](#)
- 32 Chiang, G.G. and Sefton, B.M. (2001) Specific dephosphorylation of the Lck tyrosine protein kinase at Tyr-394 by the SHP-1 protein-tyrosine phosphatase. *J. Biol. Chem.* **276**, 23173–23178 [CrossRef PubMed](#)
- 33 Cuevas, B., Lu, Y., Watt, S., Kumar, R., Zhang, J., Siminovitch, K.A. and Mills, G.B. (1999) SHP-1 regulates Lck-induced phosphatidylinositol 3-kinase phosphorylation and activity. *J. Biol. Chem.* **274**, 27583–27589 [CrossRef PubMed](#)
- 34 Stebbins, C.C., Watzl, C., Billadeau, D.D., Leibson, P.J., Burshtyn, D.N. and Long, E.O. (2003) Vav1 dephosphorylation by the tyrosine phosphatase SHP-1 as a mechanism for inhibition of cellular cytotoxicity. *Mol. Cell Biol.* **23**, 6291–6299 [CrossRef PubMed](#)
- 35 Sozio, M.S., Mathis, M.A., Young, J.A., Walchli, S., Pitcher, L.A., Wrage, P.C., Bartok, B., Campbell, A., Watts, J.D., Aebersold, R. et al. (2004) PTPH1 is a predominant protein-tyrosine phosphatase capable of interacting with and dephosphorylating the T cell receptor zeta subunit. *J. Biol. Chem.* **279**, 7760–7769 [CrossRef PubMed](#)
- 36 Stanford, S.M., Rapini, N. and Bottini, N. (2012) Regulation of TCR signalling by tyrosine phosphatases: from immune homeostasis to autoimmunity. *Immunology* **137**, 1–19 [CrossRef PubMed](#)
- 37 Sathish, J.G., Dolton, G., LeRoy, F.G. and Matthews, R.J. (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. *J. Immunol.* **178**, 330–337 [CrossRef PubMed](#)
- 38 Sathish, J.G., Johnson, K.G., LeRoy, F.G., Fuller, K.J., Hallett, M.B., Brennan, P., Borysiewicz, L.K., Sims, M.J. and Matthews, R.J. (2001) Requirement for CD28 co-stimulation is lower in SHP-1-deficient T cells. *Eur. J. Immunol.* **31**, 3649–3658 [CrossRef PubMed](#)
- 39 Bui, J.D., Uppaluri, R., Hsieh, C.-S. and Schreiber, R.D. (2006) Comparative analysis of regulatory and effector T cells in progressively growing versus rejecting tumors of similar origins. *Cancer Res.* **66**, 7301–7309 [CrossRef PubMed](#)
- 40 Lype, T., Sankarshanan, M., Mauldin, I.S., Mullins, D.W. and Lorenz, U. (2010) The protein tyrosine phosphatase SHP-1 modulates the suppressive activity of regulatory T cells. *J. Immunol.* **185**, 6115–6127 [CrossRef PubMed](#)
- 41 Johnson, D.J., Pao, L.I., Dhanji, S., Murakami, K., Ohashi, P.S. and Neel, B.G. (2013) Shp1 regulates T cell homeostasis by limiting IL-4 signals. *J. Exp. Med.* **210**, 1419–1431 [CrossRef PubMed](#)
- 42 Abram, C.L., Roberge, G.L., Pao, L.I., Neel, B.G. and Lowell, C.A. (2013) Distinct roles for neutrophils and dendritic cells in inflammation and autoimmunity in motheaten mice. *Immunity* **38**, 489–501 [CrossRef PubMed](#)
- 43 Kaneko, T., Saito, Y., Kotani, T., Okazawa, H., Iwamura, H., Sato-Hashimoto, M., Kanazawa, Y., Takahashi, S., Hiromura, K., Kusakari, S. et al. (2012) Dendritic cell-specific ablation of the protein tyrosine phosphatase Shp1 promotes Th1 cell differentiation and induces autoimmunity. *J. Immunol.* **188**, 5397–5407 [CrossRef PubMed](#)
- 44 Croker, B.A., Lawson, B.R., Rutschmann, S., Berger, M., Eidenschenk, C., Blasius, A.L., Moresco, E.M., Sovath, S., Cengia, L., Shultz, L.D. 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 [CrossRef PubMed](#)
- 45 Pao, L.I., Lam, K.-P., Henderson, J.M., Kutok, J.L., Alimzhanov, M., Nitschke, L., Thomas, M.L., Neel, B.G. and Rajewsky, K. (2007) B cell-specific deletion of protein-tyrosine phosphatase Shp1 promotes B-1a cell development and causes systemic autoimmunity. *Immunity* **27**, 35–48 [CrossRef PubMed](#)
- 46 Green, M.C. and Shultz, L.D. (1975) Motheaten, an immunodeficient mutant of the mouse. I. Genetics and pathology. *J. Heredity* **66**, 250–258
- 47 Shultz, L.D., Coman, D.R., Bailey, C.L., Beamer, W.G. and Sidman, C.L. (1984) Viable motheaten, a new allele at the motheaten locus. I. Pathology. *Am. J. pathology* **116**, 179–192
- 48 Tsui, H.W., Siminovitch, K.A., de Souza, L. and Tsui, F.W. (1993) Motheaten and viable motheaten mice have mutations in the haematopoietic cell phosphatase gene. *Nat. Genet.* **4**, 124–129 [CrossRef PubMed](#)
- 49 Shultz, L.D., Schweitzer, P.A., Rajan, T.V., Yi, T., Ihle, J.N., Matthews, R.J., Thomas, M.L. and Beier, D.R. (1993) Mutations at the murine motheaten locus are within the hematopoietic cell protein-tyrosine phosphatase (Hcph) gene. *Cell* **73**, 1445–1454 [CrossRef PubMed](#)
- 50 Shultz, L.D., Rajan, T.V. and Greiner, D.L. (1997) Severe defects in immunity and hematopoiesis caused by SHP-1 protein-tyrosine-phosphatase deficiency. *Trends Biotechnol.* **15**, 302–307 [CrossRef PubMed](#)
- 51 Minton, K. (2013) Animal models: unravelling the motheaten phenotype. *Nat. Rev. Immunol.* **13**, 306–307 [CrossRef PubMed](#)
- 52 Stromnes, I.M., Fowler, C., Casamina, C.C., Georgopoulos, C.M., McAfee, M.S., Schmitt, T.M., Tan, X., Kim, T.-D., Choi, I., Blattman, J.N. and Greenberg, P.D. (2012) Abrogation of Src homology region 2 domain-containing phosphatase 1 in tumor-specific T cells improves efficacy of adoptive immunotherapy by enhancing the effector function and accumulation of short-lived effector T cells *in vivo*. *J. Immunol.* **189**, 1812–1825 [CrossRef PubMed](#)
- 53 Yi, T., Pathak, M.K., Lindner, D.J., Ketterer, M.E., Farver, C. and Borden, E.C. (2002) Anticancer activity of sodium stibogluconate in synergy with IFNs. *J. Immunol.* **169**, 5978–5985 [CrossRef PubMed](#)
- 54 Naing, A., Reuben, J.M., Camacho, L.H., Gao, H., Lee, B.N., Cohen, E.N., Verschraegen, C., Stephen, S., Aaron, J., Hong, D., Wheler, J. and Kurzrock, R. (2011) Phase I dose escalation study of sodium stibogluconate (SSG), a protein tyrosine phosphatase inhibitor, combined with interferon alpha for patients with solid tumors. *J. Cancer* **2**, 81–89 [CrossRef PubMed](#)
- 55 Kundu, S., Fan, K., Cao, M., Lindner, D.J., Zhao, Z.J., Borden, E. and Yi, T. (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 [CrossRef PubMed](#)
- 56 Chen, L., Sung, S.-S., Yip, M.L., Lawrence, H.R., Ren, Y., Guida, W.C., Sebt, S.M., Lawrence, N.J. and Wu, J. (2006) Discovery of a novel Shp2 protein tyrosine phosphatase inhibitor. *Mol. Pharmacol.* **70**, 562–570 [CrossRef PubMed](#)
- 57 Shi, Y., Ma, I.T., Patel, R.H., Shang, X., Chen, Z., Zhao, Y., Cheng, J., Fan, Y., Rojas, Y., Barbieri, E. et al. (2015) NSC-87877 inhibits DUSP26 function in neuroblastoma resulting in p53-mediated apoptosis. *Cell Death Dis.* **6**, e1841 [CrossRef PubMed](#)
- 58 Zhang, Y.-L., Keng, Y.-F., Zhao, Y., Wu, L. and Zhang, Z.-Y. (1998) Suramin is an active site-directed, reversible, and tight-binding inhibitor of protein-tyrosine phosphatases. *J. Biol. Chem.* **273**, 12281–12287 [CrossRef PubMed](#)
- 59 Chio, C.M., Lim, C.S. and Bishop, A.C. (2015) Targeting a cryptic allosteric site for selective inhibition of the oncogenic protein tyrosine phosphatase Shp2. *Biochemistry* **54**, 497–504 [CrossRef PubMed](#)
- 60 Carter, J.D., Calabrese, G.M., Naganuma, M. and Lorenz, U. (2005) Deficiency of the Src homology region 2 domain-containing phosphatase 1 (SHP-1) causes enrichment of CD4+ CD25+ regulatory T cells. *J. Immunol.* **174**, 6627–6638 [CrossRef PubMed](#)
- 61 Sathish, J.G., Johnson, K.G., LeRoy, F.G., Fuller, K.J., Hallett, M.B., Brennan, P., Borysiewicz, L.K., Sims, M.J. and Matthews, R.J. (2001) Requirement for CD28 co-stimulation is lower in SHP-1-deficient T cells. *Eur. J. Immunol.* **31**, 3649–3658 [CrossRef PubMed](#)
- 62 Maheshwari, A., Seth, A., Kaur, S., Aneja, S., Rath, B., Basu, S., Patel, R. and Dutta, A.K. (2011) Cumulative cardiac toxicity of sodium stibogluconate and amphotericin B in treatment of kala-azar. *Pediatr. Infect. Dis. J.* **30**, 180–181 [CrossRef PubMed](#)
- 63 Insabato, L., Amelio, I., Quarto, M., Zannetti, A., Tolino, F., de Mauro, G., Cerchia, L., Riccio, P., Baumhoer, D., Condorelli, G. et al. (2009) Elevated expression of the tyrosine phosphatase SHP-1 defines a subset of high-grade breast tumors. *Oncology* **77**, 378–384 [CrossRef PubMed](#)
- 64 Mok, S.C., Kwok, T.T., Berkowitz, R.S., Barrett, A.J. and Tsui, F.W. (1995) Overexpression of the protein tyrosine phosphatase, nonreceptor type 6 (PTPN6), in human epithelial ovarian cancer. *Gynecol. Oncol.* **57**, 299–303 [CrossRef PubMed](#)
- 65 Zhang, Q., Wang, H.Y., Marzec, M., Raghunath, P.N., Nagasawa, T. and Wasik, M.A. (2005) STAT3- and DNA methyltransferase 1-mediated epigenetic silencing of SHP-1 tyrosine phosphatase tumor suppressor gene in malignant T lymphocytes. *Proc. Natl. Acad. Sci. U.S.A.* **102**, 6948–6953 [CrossRef PubMed](#)

- 66 Li, Y., Yang, L., Pan, Y., Yang, J., Shang, Y. and Luo, J. (2014) Methylation and decreased expression of SHP-1 are related to disease progression in chronic myelogenous leukemia. *Oncol. Rep.* **31**, 2438–2446 [PubMed](#)
- 67 Xu, S., Liu, X., Li, B., Zhang, Y., Yuan, J., Yuan, Q., Li, P., Yang, X., Li, F. and Zhang, W. (2009) DNA methylation regulates constitutive expression of Stat6 regulatory genes SOCS-1 and SHP-1 in colon cancer cells. *J. Cancer Res. Clin. Oncol.* **135**, 1791–1798 [CrossRef PubMed](#)
- 68 Beane, J.D., Lee, G., Zheng, Z., Mendel, M., Abate-Daga, D., Bharathan, M., Black, M., Gandhi, N., Yu, Z., Chandran, S. et al. (2015) Clinical scale zinc finger nuclease-mediated gene editing of PD-1 in tumor infiltrating lymphocytes for the treatment of metastatic melanoma. *Mol. Ther.* **23**, 1380–1390 [CrossRef PubMed](#)
- 69 Singh, H., Huls, H., Kebriaei, P. and Cooper, L.J.N. (2014) A new approach to gene therapy using Sleeping Beauty to genetically modify clinical-grade T cells to target CD19. *Immunol. Rev.* **257**, 181–190 [CrossRef PubMed](#)
- 70 Schumann, K., Lin, S., Boyer, E., Simeonov, D.R., Subramaniam, M., Gate, R.E., Haliburton, G.E., Ye, C.J., Bluestone, J.A., Doudna, J.A. and Marson, A. (2015) Generation of knock-in primary human T cells using Cas9 ribonucleoproteins. *Proc. Natl. Acad. Sci. U.S.A.* **112**, 10437–10442 [CrossRef PubMed](#)
- 71 Poirot, L., Philip, B., Schiffer-Mannioui, C., Le Clerre, D., Chion-Sotinel, I., Derniame, S., Potrel, P., Bas, C., Lemaire, L., Galetto, R. et al. (2015) Multiplex genome-edited T-cell manufacturing platform for “Off-the-Shelf” adoptive T-cell immunotherapies. *Cancer Res.* **75**, 3853–3864 [CrossRef PubMed](#)
- 72 Chen, Y.Y. (2015) Efficient gene editing in primary human T cells. *Trends Immunol.* **36**, 667–669 [CrossRef PubMed](#)
- 73 Lloyd, A., Vickery, O.N. and Laugel, B. (2013) Beyond the antigen receptor: editing the genome of T-cells for cancer adoptive cellular therapies. *Front. Immunol.* **4**, 221 [CrossRef](#)
- 74 Verbrugge, A., Ruiten, T.d., Clevers, H. and Meeyaard, L. (2003) Differential contribution of the immunoreceptor tyrosine-based inhibitory motifs of human leukocyte-associated Ig-like receptor-1 to inhibitory function and phosphatase recruitment. *Int. Immunol.* **15**, 1349–1358 [CrossRef PubMed](#)

---

Received 15 January 2016  
doi:10.1042/BST20150251