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Novel SHP-1 Inhibitors Tyrosine Phosphatase Inhibitor-1 and Analogs with Preclinical Anti-Tumor Activities as Tolerated Oral Agents

Suman Kundu,* Keke Fan,* Mingli Cao,* Daniel J. Lindner,†,‡ Zhizhuang Joe Zhao,§ Ernest Borden,*†,‡ and Taolin Yi*†

Src homology region 2 domain-containing phosphatase 1 (SHP-1) has been implicated as a potential cancer therapeutic target by its negative regulation of immune cell activation and the activity of the SHP-1 inhibitor sodium stibogluconate that induced IFN-γ+ cells for anti-tumor action. To develop more potent SHP-1-targeted anti-cancer agents, inhibitory leads were identified from a library of 34,000 drug-like compounds. Among the leads and active at low nM for recombinant SHP-1, tyrosine phosphatase inhibitor-1 (TPI-1) selectively increased SHP-1 phospho-substrates (pLck-pY394, pZap70, and pSlyp76) in Jurkat T cells but had little effects on pERK1/2 or pLck-pY505 regulated by phosphatases SHP-2 or CD45, respectively. TPI-1 induced mouse splenic IFN-γ+ cells in vitro, ~58-fold more effective than sodium stibogluconate, and increased mouse splenic-pLck-pY394 and -IFN-γ+ cells in vivo. TPI-1 also induced IFN-γ+ cells in human peripheral blood in vitro. Significantly, TPI-1 inhibited (~83%, p < 0.002) the growth of B16 melanoma tumors in mice at a tolerated oral dose in a T cell-dependent manner but had little effects on B16 cell growth in culture. TPI-1 also inhibited B16 tumor growth and prolonged tumor mice survival as a tolerated s.c. agent. TPI-1 analogs were identified with improved activities in IFN-γ+ cell induction and in anti-tumor actions. In particular, analog TPI-1a4 as a tolerated oral agent completely inhibited the growth of K1735 melanoma tumors and was more effective than the parental lead against MC-26 colon cancer tumors in mice. These results designate TPI-1 and the analogs as novel SHP-1 inhibitors with anti-tumor activity likely via an immune mechanism, supporting SHP-1 as a novel target for cancer treatment. The Journal of Immunology, 2010, 184: 000–000.

Activating immune cells for cancer treatment has been investigated intensively for decades with certain degrees of success and holds promise for increasing cancer cures (1). For instance, IL-2 is a potent immune cell activator capable of inducing complete responses durable for more than 10 years in melanoma or renal cancer patients (2, 3). However, its application is restricted by high toxicity and the limitation of efficacy within a subpopulation of patients (4). With increased mechanistic understanding of immune cell signaling (5, 6), alternative approaches for immune cell activation could be exploited (7–9) and might lead to more efficacious and tolerated cancer treatments.

Targeting protein tyrosine phosphatases (PTPs) that negatively regulate immune cells with inhibitors is an attractive strategy. Like protein tyrosine kinases that have been targeted successfully with inhibitors for cancer treatment (10–12), PTPs are key regulators of intracellular signaling and potential targets for developing novel cancer therapeutics (13–17). Specifically, several PTPs have been identified as negative regulators of immune cells or cytokines and might be targeted to improve immunotherapy and cytokine therapy (14, 17–21). In addition, oncogenic PTPs play a causal role in oncogenesis (22–25) or metastasis (26) and could be targeted to block their pathogenic activities. Further, tumor suppressor PTPs have also been reported (27) and might be exploited through activating downstream signaling molecules. However, it remains to be established that targeting PTPs could be an effective and safe anti-cancer strategy. Few PTP inhibitors with preclinical anti-tumor activity and clinical potential have been reported (13–17).

Anti-cancer potential of activating immune cells via targeting negative regulatory PTPs has been suggested by the anti-tumor actions of Src homology region 2 domain-containing phosphatase 1 (SHP-1) inhibitory sodium stibogluconate (SSG). SSG is an anti-leishmanial drug with previously undefined mechanism of action (28). SSG selectively inhibited the PTP SHP-1 (29), which negatively controls the activation of immune cells (14, 21, 30) essential for anti-tumor immunity (1) and downregulates the signaling of anti-tumor cytokines (31–33). Consistent with targeting SHP-1, SSG had anti-renal tumor activity mediated via activating Th1 cells (IFN-γ+ T cells) in mice when combined with IL-2 (34) and required IFN-γ for anti-tumor action (35). SSG also synergized with IFN-α in mouse models to eradicate melanoma tumors (36) and to inhibit the growth of prostate cancer tumors (37). This preclinical evidence provided a basis for moving SSG into clinical trials (NCT00311558, NCT00629200, and NCT00498979), one of which has completed enrollment and reported evidence of augmenting anti-tumor immunity by SSG in cancer patients (38). These results together support the development of refined small molecule SHP-1 inhibitors as more effective cancer therapeutics.

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Abbreviations used in this paper: PTP, protein tyrosine phosphatase; SHP, Src homology region 2 domain-containing phosphatase; SSG, sodium stibogluconate; TCL, total cell lysate; TPI, tyrosine phosphatase inhibitor.

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In this work, TPI-1 and its analogs were identified as more effective than SSG in SHP-1 inhibition, immune cell activation, and anti-tumor activity. Our results provide important evidence supporting targeting SHP-1 to activate immune cells as an anti-cancer strategy and designate TPI-1 and the analogs as promising leads and a valuable platform for further investigation.

Materials and Methods

Cells, cell culture, and reagents

Recombinant protein of SHP-1 catalytic domain was described previously (39). Fluorescence substrate 6, 8-difluoro-4-methylumbelliferyl phosphate was purchased (Sigma-Aldrich, St. Louis, MO). SSG, recombinant SHP-2, and MKP1 were reported previously (34, 36, 37). Human and mouse IFN-γ ELISPOT Kit (R&D Systems, Minneapolis, MN), CD4+ Cell Intracellular IFN-γ Detection Kit (BD Bioscience, San Jose, CA), and CD8+ Cell Intracellular IFN-γ Detection Kit (BD Bioscience) were purchased from commercial sources. Jurkat human T cell line (40), B16 murine melanoma cell line (41) (American Type Culture Collection, Manassas, VA), MC-26 murine colon cancer cell line (42), and K1735 murine melanoma cell line (43) (Institutional tumor core) were maintained in DMSO culture medium supplemented with 10% FCS. Abs against pLck-pY394 (Cell Signaling (43)) (Institutional tumor core) were maintained in DMSO culture medium supplemented with 10% FCS in the absence or presence of designated agents for 4 h. The splenocytes were cultured in RPMI 1640 medium supplemented with 10% FCS. tRNA was purchased (Molecular Probes, Eugene, OR). SSG, recombinant SHP-2, and MKP1 were reported previously (34, 36, 37).

Evaluation of chemical compounds by PTP assays

A rapid SHP-1 PTP assay was developed for screening the compounds in a commercial library of 34,000 drug-like small chemicals (Chembridge, MA) and for evaluating lead compounds and analogs. Briefly, compounds were plated in 96-well plates (353072; BD Falcon, San Jose, CA) and plated with recombinant SHP-1 protein (0.1 μg/well) in 90 μl HEPES buffer (50 mM HEPES, pH 7.5, 150 mM NaCl, 1 mM EDTA, 0.2 mM DTT, and 0.1 mg/ml BSA). The plates were incubated at room temperature for 10 min prior to addition of fluorescence substrate. A 6, 8-difluoro-4 methylumbelliferyl phosphate (40 μM stock in HEPES buffer, 10 μM/well) to initiate PTP reaction. On completion of PTP reaction at room temperature for 1 h in darkness, fluorescence signal of individual wells were recorded using a Victor Multilabel Counter (Victor, CA). They were compared with that of control SHP-1 PTP reaction (≈10,000 U fluorescence signal) in the absence of any compound (100%) for calculating relative SHP-1 inhibition induced by the compounds after subtracting the background signal (≈500 U fluorescence signal) of the substrate. The activities of lead compounds on recombinant SHP-2 or MKP1 were evaluated similarly. Analogs of lead compound TPI-1 from the library were identified via searching pubchem databases of ~1 million compounds based on structural similarities and purchased from commercial sources (Chembridge, MA). Their chemical features of drugability were extracted from pubchem databases.

Induction and detection of cellular protein tyrosine phosphorylation in Jurkat cells

Jurkat cells in culture medium (3 × 10⁶ cells/ml) were treated with agents for designated times at room temperature. After brief centrifuging in a microfuge (4000 rpm, 2 min), the cell pellet was lysed on ice for 30 min in 100 μl cold lysis buffer (1% NP40, 50 mM Tris, pH 7.4, 150 mM NaCl, 20 mM NaF, 0.2 mM NaNVO₃, and 1 mM NaMO₄) containing a mixture of proteinase inhibitors (Sigma-Aldrich, St. Louis, MO, 1 tablet/10 ml). The lysates were cleared by centrifuging (14,000 rpm, 10 min) in a microfuge at 4°C to remove insoluble parts, mixed with equal volume of 2 × SDS-PAGE sample buffer, boiled for 5 min and analyzed (~3 × 10⁶ cells/well) by SDS-PAGE/Western blotting as described previously (46, 47). Relative intensities of phosphosyrosine bands were quantified through densitometry analysis.

Induction and quantification of mouse and human IFN-γ cells

For induction of mouse primary IFN-γ cells, splenocytes from female mice (129, 3–5 wk old, Taconic Farms, Germantown, NY) were prepared as reported previously (34) according to an established protocol approved by the Institutional Animal Care and Use Committee of the Cleveland Clinic. The splenocytes were cultured in RPMI 1640 medium supplemented with 10% FCS in the absence or presence of designated agents for 16 h in flat-bottom 96-well plates coated with a mAb specific for mouse IFN-γ (mouse IFN-γ ELISPOT Kit, R&D Systems). The plates were then processed for in situ detection of IFN-γ cells by ELISA following the manufacturer’s procedure. Scanning and counting of IFN-γ+ cells in the plates were accomplished using an automatic ELISPOT reader with Immunospot2 software (Cellular Technology, Shaker Heights, OH).

For induction of human primary IFN-γ cells, hepatopurified peripheral blood samples were obtained by vein-puncture from healthy volunteers following an established protocol approved by the Institutional Review Board of Cleveland Clinic. To mimic in vivo drug-exposure, human peripheral blood samples were directly treated with different agents without preseparation of WBCs from other blood components. Blood samples (0.1 ml/sample) were mixed with the agents, incubated at 37°C for 4 h, diluted with five volumes of hypotonic solution (10 mM Tris, pH 7.4; 10 mM NaCl) to lyse RBC and centrifuged to pellet WBCs. The pellets were washed with hypotonic solution one time, resuspended in RPMI 1640 medium (10% FCS), and used for ELISPOT assays (Human IFN-γ ELISPOT Kit, R&D System) to quantify human IFN-γ+ cells as outlined previously.

Animals and animal studies

For in vivo induction of pLck-pY394 and IFN-γ+ cells in mice, C57BL/6J mice (~8 wk-old, female, Taconic Farms) were treated with PBS or TPI-1 (~1 or 3 mg/kg, s.c.) for 4 d. Spleens were harvested 1 h posttreatment on day 4 and processed into splenocytes, which were used for assessing pLck-pY394 levels by SDS-PAGE/Western blotting and for quantification of IFN-γ+ cells by ELISPOT assays. Mice were also treated with TPI-1 (~10 mg/kg, daily, s.c., n = 2) to evaluate the toxicity of the compounds in vivo.

Results

Identification of SHP-1 inhibitor TPI-1 from a library of drug-like small chemicals

To identify novel SHP-1 inhibitors, compounds (~34,000) in a chemical library were screened for candidates capable of inhibiting recombinant SHP-1 in PTP assays. Twenty-nine compounds were identified (data not shown) and designated as leads. Given their intended use for targeting SHP-1 in immune cells, the leads were evaluated for the capacity to inhibit cellular SHP-1 and thus increase SHP-1 substrate pLck-pY394 (49) in Jurkat T cells, in which the PTP and the substrate (49) were reported (50). Lead compound no. 5 was the most active among the leads and increased pLck-pY394 levels ~10-fold under the experimental conditions (Fig. 1A). It was named as TPI-1 (Fig. 1B).

As an initial step to evaluate its potential for further development, TPI-1 was assessed for toxicity in vitro and in vivo. TPI-1 had little impact on Jurkat cell growth in vitro during 6 d coculture (Fig. 1C) and was tolerated by mice as a daily treatment at 10 mg/kg for 2 wk (Fig. 1D). Consistent with this indication of limited off-target effects for the lead, TPI-1 effectively in inhibited recombinant SHP-1 (IC₅₀ <0.1 μg/ml or 40 nM) but had limited impact on recombinant MKP1 (IC₅₀ > 1 μg/ml) or SHP-2 (IC₅₀ > 0.1 μg/ml) under the experimental conditions (Fig. 1E). TPI-1 was therefore chosen for further evaluation.

TPI-1 selectively increases SHP-1 phospho-substrates in Jurkat T cells at low nanomolar

To assess the potency and selectivity of TPI-1 for cellular SHP-1 in immune cells, the impacts of TPI-1 at a dose range on phospho-substrates of SHP-1, SHP-2, and CD45 in human Jurkat T cells were determined. Expression of SHP-1, SHP-2, and CD45 in Jurkat cells were reported previously (51, 52) and verified (data not shown).
TPI-1 was effective starting at 10 ng/ml in increasing SHP-1 phospho-substrates pLck-pY394 (49), pZap70 (53) and pSlp76 (54) in Jurkat cells (Fig. 2A). The pLAT, which functions downstream from pLck during T cell activation (55), was also elevated (Fig. 2A). This TPI-1 action was selective because it failed to equally affect phospho-proteins pERK1/2 (56, 57) and pSlp76/pY505 (58) that are regulated by CD45 and SHP-2, respectively. TPI-1 selectively increases SHP-1 phospho-substrates in Jurkat cells at low nanomolar levels. Jurkat cells were untreated or treated with TPI-1 (A–C) or nonspecific phosphatase inhibitor pervanadate (D) at various doses for 10 min. TCL of the cells were prepared and analyzed by SDS-PAGE/Western blotting with Abs as indicated.

These results together provided evidence that TPI-1 selectively inhibited cellular SHP-1 in Jurkat T cells at low nanogram/milliliter concentrations.

**TPI-1 increases IFN-γ** cells in mouse spleen and human peripheral blood in vitro

IFN-γ is a Th1 cytokine expressed in activated anti-tumor immune cells (59, 60), in which SHP-1 is a key negative regulator (18). IFN-γ cells were induced by SSG in its anti-Renca tumor action (34) and in human peripheral blood samples in vitro (35). As a further step to assess TPI-1, the capacity of the SHP-1 inhibitor to induce primary IFN-γ cells in mouse spleenocyt and human peripheral blood in vitro were evaluated in comparison with SSG.

TPI-1 markedly induced IFN-γ cells in mouse splenocytes (Fig. 3A) and human peripheral blood (Fig. 3C). IFN-γ cells were increases in splenocytes treated with TPI-1 at 1 µg/ml (∼14-fold), 3 µg/ml (∼26-fold), 10 µg/ml (∼17-fold), and 30 µg/ml (∼10-fold) (Fig. 3A). In contrast, SSG induced maximal increase ∼3-fold at its optimal dose (20 µg/ml (Fig. 3B)). IFN-γ cells in human peripheral blood were also induced by TPI-1 (maximal 20-fold at 8 µg/ml (Fig. 3C), more effective than SSG (∼2-fold at 20 µg/ml (Fig. 3D)).

These results demonstrated that TPI-1 was a potent inducer of mouse and human primary IFN-γ cells in vitro. When compared with SSG for maximal induction at a comparable dose, TPI-1 was more effective in inducing IFN-γ cells in mouse splenocytes (∼58-fold) and human peripheral blood (∼20-fold).

**TPI-1 increases mouse spleen pLck-pY394 and IFN-γ+ cells in vivo**

Given the TPI-1 capacity to induce phosphorylation of SHP-1 substrates and to induce IFN-γ+ cells in vitro, we next determined whether TPI-1 possessed similar activities in vivo as well. Spleens from mice untreated or treated with TPI-1 were harvested for evaluation of pLck-pY394 levels and IFN-γ+ cells in splenocytes.

Splenocyte pLck-pY394 was detectable in untreated mice (Fig. 4A, lane 1) and was further increased (∼3.3-fold, Fig. 4B) in mice treated with TPI-1 at ∼3 mg/kg body weight (Fig. 4A, lane 3). Spleen IFN-γ+ cells were also increased ∼3-fold (Fig. 4C) in mice treated with a comparable dose of TPI-1. At a lower dose (1 mg/kg body weight), TPI-1 had only a minor effect on pLck-pY394 (Fig. 4A, 4B) under the experimental conditions. The effects of the low dose of TPI-1 on spleen IFN-γ+ cells were not determined.

Consistent with its in vitro activity, TPI-1 thus also increased pLck-pY394 and IFN-γ+ cells in mice, demonstrating that the compound was effective in vivo as well. The reason for the lower levels of TPI-1-induced pLck-pY394 and IFN-γ+ cells in vivo (Fig. 4) in comparison with those in vitro (Figs. 2, 3) have not been determined and could be resulted from TPI-1 clearance or metabolism in vivo.

**TPI-1 inhibits the growth of B16 melanoma tumors in mice as a tolerated single agent**

TPI-1 might have anti-cancer potential based on its induction of IFN-γ+ cells critical in anti-tumor immunity. This potential was investigated by assessing its effects on B16 melanoma tumors in mice in comparison with IL-2 or SSG. The melanoma model (41) was chosen for optimal detection of TPI-1 anti-tumor effects via immunity in the absence of direct drug actions on cancer cells, as TPI-1 had little direct toxicity against B16 cells in culture at doses up to 10 µg/ml (Fig. 5A). Like advanced human melanoma, B16 melanoma forms aggressive tumors in mice that are resistant to chemotherapeutics and poorly antigenic (61).
B16 tumors grew aggressively in control mice (Fig. 5B), which had to be terminated by the third week due to large tumor burden and tumor ulceration. Growth of B16 tumors in mice treated with oral TPI-1 was slower and induced ~83% of growth inhibition of B16 tumors compared ($p < 0.002$) with that of the control (Fig. 5B).

Supporting an immune mechanism of action, TPI-1 failed to inhibit B16 melanoma tumors in athymic nude mice with T cell deficiency under comparable experimental conditions (Fig. 5C). To evaluate its effectiveness at a different dose and via an alternative route of delivery, TPI-1 (1 mg/kg, s.c.) also showed significant activity ($p < 0.01$) against B16 tumors in mice (Fig. 5D). TPI-1 was tolerated: all the mice in the study survived to the end of study with comparable body weight (Fig. 5F) and no obvious abnormalities in behaviors or gross anatomy (data not shown). Similar data were derived in a repeated experiment, in which the compound inhibited tumor growth (Fig. 5G) and prolonged survival of melanoma tumor mice (Fig. 5F). The optimal dose for TPI-1 has not been determined although oral TPI-1 at 30 mg/kg was tolerated by mice in a pilot experiment (data not shown).

B16 tumors were not responsive to an SSG treatment (Fig. 5E), which was effective against several other types of tumors in mice (34, 36, 62). The growth of the tumors was inhibited (~61%) by high dose IL-2 (3 × 10^5 IU, twice daily) (Fig. 5H), an FDA-approved front line treatment for melanoma and capable of inducing durable complete response (3). At a lower dose (1 × 10^5 IU, twice daily), IL-2 had little effect on B16 tumor growth (data not shown).

These results demonstrated an anti-B16 melanoma activity for TPI-1 that was more effective than SSG but comparable to IL-2 under the experimental conditions.

Identification of TPI-1 analogs with correlated activities in SHP-1 inhibition, IFN-γ* cell induction and anti-tumor action

Encouraged by the above data, 10 chemical analogs (TPI-1a1–10) of TPI-1 were identified and evaluated for improved activities and for mechanistic insights.

Analog TPI-1a1–5 effectively increased SHP-1 substrate pLck-pY394 in Jurkat cells in contrast to the inactive TPI-1a6–10 (Fig. 6B). TPI-1a1–2 data not shown). Similar to their parental compound, the active TPI-1a1–5 were also found to have excellent druggability based on Lipinski’s “Rule of Five” and Extensions (63) (Table I). TPI-1a1–5 and the representative inactive TPI-1a10 (Fig. 6A) were therefore further evaluated.

The active analogs TPI-1a1–5 were all capable of inhibiting recombinant SHP-1 in vitro, whereas TPI-1a10 was not active under comparable conditions (Fig. 6C). The active analogs also induced IFN-γ* cells in mouse splenocytes in vitro and ~2- to 4-folds more effective than the parental TPI-1 at the dose range of 1 ng–1000 ng/ml (Fig. 6D). TPI-1a10 failed to significantly induce IFN-γ* cells under comparable conditions at doses from 1 ng–10 μg/ml (data not shown).

Two of the active analogs (TPI-1a2 and TPI-1a4) and the inactive analog L5a10 were assessed for activities in syngeneic mouse tumor models of B16 melanoma (41) or MC-26 colon cancer (42). TPI-1a2 and TPI-1a4 inhibited B16 tumor growth significantly ($p < 0.01$) (Fig. 7A, 7B), whereas TPI-1a10 was not active (Fig. 7C). TPI-1a4 as a single oral agent inhibited the growth ($p = 0.04$) of MC-26 tumors (Fig. 7E), against which TPI-1 had modest but insignificant activity ($p = 0.32$) (Fig. 7D). The compounds were tolerated as indicated by the viability of the treated mice (data not shown) and the comparable mouse body weights at the end of the experiment (Fig. 7F).

The anti-tumor activity of TPI-1a4 was further evaluated against the K1735 murine melanoma (43). Unlike the B16 melanoma from a spontaneous tumor (41), K1735 is similar to human melanoma in that it was UV-induced (43) and harbors the common N-Ras mutation (64). TPI-1a4 as a single oral agent completely inhibited the growth ($p < 0.01$) of 4-d established K1735 tumors in mice (Fig. 7G). In contrast, K1735 tumors grew aggressively in the control mice (Fig. 7G), which were moribund with large tumor burden by day 21. TPI-1a4 was tolerated by mice that maintained steady body weight (Fig. 7H). It had little effect on K1735 cell growth in culture (Fig. 7I).

Taken together, these data demonstrated correlated activities in SHP-1 inhibition, IFN-γ* cell induction and anti-tumor effects in mice for TPI-1 and its analogs, suggesting that the compounds target SHP-1 to induce IFN-γ* cells for anti-tumor action. In addition, the apparently improved activities of the analogs suggested feasibility of further refinement.

**Discussion**

Based on our prior finding of SSG as an SHP-1–inhibitory compound (29) with anti-tumor activity that was mediated significantly via IFN-γ* cells (34, 35), we sought to identify novel and more potent SHP-1 inhibitors as potential anti-cancer agents.

Our current work demonstrated that TPI-1 was a potent and selective SHP-1 inhibitor effective at low nanomolar levels (Figs. 1, 2). TPI-1 inhibited recombinant SHP-1 with IC_{50} at ~10 ng/ml (Fig. 1) and increased SHP-1 phospho-substrates pLck-pY394, pZap70, and pSlp76 in Jurkat T cells starting at 10 ng/ml (Fig. 2). Its selectivity was indicated by its reduced effectiveness for other
PTPs in vitro and its limited impact on cellular phospho-proteins regulated by CD45 or SHP-2 (Figs. 1, 2). Interestingly, TPI-1 was effective on cellular SHP-2 only at 1 μg/ml (Fig. 2), despite its lower IC50 (0.1 μg/ml) for recombinant SHP-2 (Fig. 1). Assessment of cellular IC50s based on intracellular substrate phosphorylation has been common for characterizing protein tyrosine kinase inhibitors (65, 66), including those approved for clinical use (67), and is believed more predictive for intracellular inhibition than data from recombinant enzymes. Indeed, several of our initial 29 lead compounds from the library failed to increase SHP-1 phospho-substrate in Jurkat T cells (Fig. 1A), indicating their lack of activity for the cellular enzyme although they were inhibitory for the recombinant phosphatase.

Consistent with its capacity to inhibit SHP-1, TPI-1 was an effective inducer of IFN-γ+ cells in mouse splenocytes and in human peripheral blood in vitro and ~10–20 times more effective than SSG (Fig. 3). TPI-1 was also active in vivo and increased pLck-pY394 and IFN-γ+ cells in mice (Fig. 4). The induction of IFN-γ+ cells by TPI-1 was not unexpected given its capacity to rapidly increase cellular pLck and pZap70, etc. (Fig. 2) that mediate activating signals in immune cells (6). Indeed, IFN-γ+ cells could be induced rapidly by stimulation with anti-CD3 Ab that activates the Lck and Zap70 kinases (6, 68, 69). Thus, TPI-1–induced pLck and pZap70 might be sufficient as substitutions for extracellular stimuli in inducing IFN-γ+ cells. Moreover, induction of IFN-γ+ cells by SHP-1 inhibitors is also supported by the increases of IFN-γ+ cells.
Table 1. Druggability of the lead compounds

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<th>Characteristics</th>
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<th>a2</th>
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*Note: Any compound that satisfies all five rules is predicted to have high druggability. The values represent the percentage of compounds that pass each rule.*

Inhibited B16 tumors (Fig. 5D) and prolonged survival of mice bearing B16 tumors when administered s.c. (Fig. 5G).

The anti-B16 tumor activity of TPI-1 was comparable to those of IL-2 under the experimental conditions (Fig. 5B, 5D, 5G, 5H). Although IL-2 induces durable complete responses in some patients, its clinical efficacy and application are limited by toxicity (2–4, 71, 72). Potential therapeutics derived from TPI-1 might be less toxic and more convenient as an oral agent. TPI-1 also represents an improvement over SSG that was inactive for B16 tumors (Fig. 5E). Additional studies of mouse tumor models will help to define the mechanism of TPI-1 anti-melanoma action and to assess the optimal TPI-1 capacity to cure established tumors at higher doses or in combination with other agents. In this regard, it is worth noting that TPI-1 inhibitory activity against B16 tumors (Fig. 6B) was achieved using a TPI-1 dose (~3 mg/kg) lower than its maximal tolerated dose (>10 mg/kg) (Fig. 2C).

At its effective doses, TPI-1 apparently acted predominantly against SHP-1 in immune cells with little or inconsequential interactions with other PTPs or cellular signaling molecules. This was indicated by the observation that TPI-1 at effective SHP-1 inhibition doses (10–100 ng/ml) did not significantly affect pERK1/2 or pLck-pY505 that were regulated SHP-2 and CD45 PTPs, respectively (Fig. 3C). It was further supported by the tolerance of immune cells and mice as a whole to the compound during its action to increase SHP-1 phospho-substrates, induce IFN-γ cells and inhibit B16 tumor growth in mice. If other PTPs were also affected by TPI-1, it did not prevent TPI-1 from activating immune cells for anti-tumor action and might contribute to the effects due to functional overlaps with SHP-1. The potential value and clinical efficacy of a tolerated SHP-1 inhibitor with desired activity but targeting multiple molecules were indicated by the three Food and Drug Administration-approved kinase inhibitors (imatinib, sorafenib, and sunitinib) that all target multiple kinases.
30. Lorenz, U. 2009. SHP-1 and SHP-2 in T cells: two phosphatases functioning at different stages of their anti-tumor action and for mechanistic analyses.
31. In summary, our work in this study has identified a novel class of small molecule SHP-1 inhibitors that were more potent than SSG and had significant preclinical anti-tumor activities likely via activating immune cells. Their translational potential was suggested by tolerance in mice, oral availability, excellent druggability and capacity to activate human immune cells. Further evaluation of these leads is warranted and could lead to promising candidates for assessing therapeutic potential for cancers and other indications.

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Disclosures
Patents for TP1-1 and analogs are pending (T.Y.).

References


