1521-0111/93/6/575–580$35.00
MOLECULAR PHARMACOLOGY

https://doi.org/10.1124/mol.117.110908
Mol Pharmacol 93:575–580, June 2018

Inhibition of CD45 Phosphatase Activity Induces Cell Cycle Arrest and Apoptosis of CD45$^+$ Lymphoid Tumors Ex Vivo and In Vivo

Michael Perron and H. Uri Saragovi

Lady Davis Institute–Jewish General Hospital and Department of Pharmacology and Therapeutics, McGill University, Montreal, Quebec, Canada

Received October 20, 2017; accepted March 14, 2018

ABSTRACT

Src-family kinases (SFK) govern cellular proliferation of bone marrow–derived cells. SFKs are regulated by the protein tyrosine phosphatase enzymatic activity of CD45. All lymphoid cells express CD45, but only proliferating cells are dependent on CD45 activity. We postulated that compound 211 (2-[(4-acetylphenyl)amino]-3-chloronaphthoquinone), a selective inhibitor of CD45 phosphatase activity, could preferentially affect actively proliferating cells but spare resting lymphoid cells. Compound 211 inhibited CD45 and induced inappropriate SFK signaling, leading to a G2/M cell cycle arrest and apoptotic cell death. CD45$^+$ cell lines were sensitive to compound 211 cytotoxicity at low micromolar LD_{50} while control CD45$^-$ cell lines and CD45$^+$ resting primary T cells were spared any toxicity. In two syngeneic tumor models in vivo, compound 211 delayed the growth of established primary tumors and reduced tumor metastasis without causing depletion of resting T cells. This work validates targeting CD45 phosphatase enzymatic activity, which may be a druggable target for cancer therapy.

Introduction

CD45 protein tyrosine phosphatase (PTP) is a master regulator of Src-family kinases (SFK), key components of B-cell and T-cell signaling pathways (Pingel and Thomas, 1989; Mustelin and Altman, 1990; Trowbridge and Thomas, 1994; Justement, 1997; Hermiston et al., 2003). CD45 can activate or inactivate SFKs Fyn (in B cells) and Lck (in T cells) (McNeill et al., 2007) by modifying their phosphotyrosine (p-Tyr) residues.

Lck pTyr-505 is a phosphotyrosine that inhibits Lck activity, and its dephosphorylation by CD45 primes Lck for activation. Full Lck activation requires phosphotyrosine pTyr-394, and dephosphorylation by CD45 brings Lck back to the primed state. Hence CD45 acts as a rheostat regulating Lck states from inhibited to primed, and from activated to primed or to inhibited. Lck that is properly regulated by CD45 governs the proliferation and maturation of T cells (Shiroo et al., 1992; Cahir McFarland et al., 1993), whereas dysregulated Lck can lead to cell death of naive T cells and thymocytes or to cancer (Alexander, 2000).

To control the growth of leukemias and lymphomas, antibodies directed to the extracellular domain of CD45 have been used to eliminate CD45$^+$ cells but have had limited clinical efficacy (Hamaguchi et al., 2001; Nemecek and Matthews, 2002; Pagel et al., 2003). One problem is that anti-CD45 monoclonal antibodies (mAb) target all CD45$^+$ cells, regardless of whether CD45 activity is functionally essential to that cell. Both resting and rapidly proliferating lymphoid cells are CD45$^+$ and are targeted by anti-CD45 mAb, causing undesired "on-target" effects upon nonproliferating cells.

We proposed using inhibitors of CD45 enzymatic activity rather than targeting the CD45 protein. The principle is that sparing normal resting lymphoid cells (which do not require CD45 activity) while targeting rapidly proliferating lymphoid cells (which do require CD45 activity) would be beneficial for suppressing the SFK-dependent growth of lymphoid cancers. The translational rationale is that SFKs are validated targets for cancer therapy (Zhao et al., 2008; Harr et al., 2010), and inhibitors of CD45 enzymatic activity would impair the SFK signals required by proliferating cells.

As a challenge to this hypothesis, PTPs have a general reputation of being "undruggable" (Blaskovich, 2009; Stanford and Bottini, 2017). In addition, inhibitors of CD45 would have to be highly selective to avoid inhibition of other PTPs, which could compromise safety (Labbé et al., 2012) and the interpretation of results, as nonspecific PTP inhibitors (e.g., oxidative agents targeting catalytic cysteine residues) cause cellular transformation and are pro-oncogenic (O'Shea et al., 1992; Volarević et al., 1992).

Allosteric inhibitors are generally deemed to achieve selectivity (Hardy and Wells, 2004; Labbé et al., 2012; Chio et al., 2015) by circumventing the highly conserved orthosteric

This work was supported by a grant from Canadian Institutes of Health Research (CIHR) to H.U.S.
https://doi.org/10.1124/mol.117.110908.

ABBREVIATIONS: Compound 211, 2-[(4-acetylphenyl)amino]-3-chloronaphthoquinone; FACS, fluorescence-activated cell sorter; HBSS, Hanks' balanced salt solution; mAb, monoclonal antibody; MTT, tetrazolium salt reagent 4-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide; PI, propidium iodide; PTP, protein tyrosine phosphatase; p-Tyr, phosphotyrosine; SFK, Src-family kinases.

catalytic domains in enzymes. Previously, we reported that small-molecule compound 211 (2-[(4-acetylphenyl)amino]-3-chloronaphthoquinone) was a selective noncompetitive allosteric CD45 inhibitor with IC_{50} 0.29 μM (Perron et al., 2014) that did not inhibit 10 related PTPases. Compound 211 was effective in vivo at suppressing T-cell responses in a delayed type hypersensitivity inflammatory model without statistically significant toxicity to the normal immune system (Perron et al., 2014). Here we report the use of compound 211 in a therapeutic cancer paradigm in vivo to prevent the growth and metastasis of established lymphoid tumors. To our knowledge this is the first proof-of-concept cancer therapy study validating CD45 enzymatic activity for cancer therapy.

Materials and Methods

CD45 Inhibitor. Compound 211 was purchased from ChemBridge (San Diego, CA). Compound 211 is a selective inhibitor of CD45 phosphatase (IC_{50} 0.29 μM); it does not affect many related PTPs, including protein tyrosine phospatase-1B, Src-homology 2 domain-containing protein-1, MEK-kinase phosphatase (a dual-specificity phosphatase), leukocyte common antigen–related, protein tyrosine phosphatase-σ , PEST-enriched phosphatase, protein tyrosine phosphatase proline-glutamic acid-serine-threonine rich, or lymphoid phosphatase (LYP or PTPN22) (Perron et al., 2014).

Cell Culture. EL4 mouse T lymphocytes (American Type Culture Collection TIB-39), $CD45^+$ Jurkat T cells and $CD45^-$ Jurkat T-cell variant J.1 human leukemic cells, Ly-1 and Toledo diffuse large B cell lymphomas (a gift of Koren Mann, McGill University, Montreal, Quebec), 5C3 hybridoma of a normal B-cell fused to SP20 myeloma and producing anti-TrkA mAb (LeSauteur et al., 1996), and KB human nasopharyngeal cancer cell lines were maintained in RPMI 1640 (Wisent Bioproducts, Saint-Jean-Baptiste, Quebec, Canada) culture media supplemented with 5% fetal bovine serum, 10 mM HEPES, penicillin (10 U/ml) and streptomycin (100 U/ml), and 2 mM glutamine (Wisent Bioproducts). Human embryonic kidney 293 (HEK293) epithelial cells were cultured in Dulbecco's modified Eagle's medium (Wisent Bioproducts) with 10% fetal bovine serum, 5 mM HEPES, penicillin and streptomycin (100 U/ml), and 2 mM glutamine.

Cell Viability Assays. Cells were plated in 96-well plates to achieve logarithmic growth in untreated control wells. Cells were plated and treated for 24 or 48 hours as indicated. Cell metabolism was quantified using the tetrazolium salt reagent 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT; Sigma-Aldrich, St. Louis, MO), an assay that correlates with cell replication and viability (Saragovi et al., 1995). Optical density readings of MTT were measured spectrophotometrically at 595 nm. Apoptosis assays and visual inspection of the cultures for cell morphologic features also provided evidence of cell death.

Biochemical Analyses. The cells were incubated for 3-hours with 0.5 μM compound 211 or vehicle. The cells were collected, and NP40 detergent lysates were prepared (Perron et al., 2014) and immunoprecipitated using rabbit antibodies against total Lck protein. Samples were Western blotted using mouse monoclonal anti-pLck Y394. Immunoblots were developed by chemiluminescence, and quantification was performed via densitometric analysis (n = 3 independent assays).

Determination of Apoptosis and Cell Cycle Analysis. EL4 cells were plated at 1×10^6 cells/well in six-well plates, and they were either left untreated or treated with 0.5 μM compound 211 for 3, 6, 15, or 24 hours. The cells were then washed in cold fluorescence-activated cell sorter (FACS) buffer (Hanks' balanced salt solution [HBSS] containing 0.5% bovine serum albumin and 0.05% sodium azide), collected in polystyrene tubes, and incubated on ice with 5 μl Annexin V-FITC (BioVision, Milpitas, CA), 5 μl propidium iodide solution (BioVision), or both for 15 minutes at 4°C. Fluorescence was then quantified by flow cytometry using a FACSCalibur machine (BD Biosciences, San Jose, CA). For cell cycle analysis, cells were permeabilized with cooled 95% ethanol and stained with propidium iodide before flow cytometry analysis in hypotonic FACS buffer (0.1% sodium citrate, 0.05% Triton-X).

Tumor Models In Vivo. All animal protocols were approved by the McGill University Institutional Animal Care and Use Committee. Six-week old female C57/BL6 mice or Balb/c mice (Charles River Laboratories, Hollister, CA) were used.

EL4 cells ($2–4 \times 10^5$, depending on the experiment) were injected subcutaneously into the left flank of syngeneic C57/BL6 mice at day 0. By day 5 a palpable tumor mass formed. The mice then were randomized into two groups (n = 7 per group) and injected daily intraperitoneally with either 3 mg/kg compound 211 or vehicle (40% dimethylsulfoxide/60% HBSS). The primary tumor was measured on days 10, 12, 14, and 18 with a digital caliper by an investigator blinded to the treatment. The data were analyzed by use of this equation: Volume (mm^3) = 0.5 × Width × $(Length)^2$. Experiments were repeated three times independently.

After euthanasia, mice were dissected and inspected for metastasis. Axillary lymph nodes contralateral to the site of primary tumor were removed and measured via calipers. The lymph node volume was calculated from the diameter using the equation $V = (4\pi r^3)/3$, and the measurements were standardized to normal lymph node volume. The tissues were studied by immunohistochemistry using anti-GD2 mAbs (a ganglioside marker of EL4 tumor cells), as described by Tong et al. (2010).

We injected 5C3 cells (1×10^6) intraperitoneally into primed syngeneic Balb/c mouse on day 0, where they grew as ascites. Abdominal swelling was palpable at day 4, at which point the mice were randomized (n = 8 per group) and injected every other day (days 4, 6, 8, 10) intraperitoneally either with 3 mg/kg compound 211 or vehicle (40% dimethylsulfoxide/60% HBSS). Ascites fluid was collected from each mouse on day 11 to measure the 5C3 cell numbers and 5C3 mAb production. Quantification of tumor cells was performed via cell counting. Quantification of 5C3 mAb titers was performed via testing the binding of serial dilutions on TrkA-expressing cells by flow cytometric analyses, as previously described elsewhere (LeSauteur et al., 1996).

Statistical Analyses. Ex vivo results were compared by t test, and error bars represent S.E.M. Statistical significance is indicated in the graphs or legends. The reported LD_{50} is taken from nonlinear regression of n = 3 independent cell death experiments (each assay with six replicates) ± S.D.

Results

CD45 Inhibitor Compound 211 Induces Cell Death in $CD45^+$ Cells. We have reported that compound 211 (Fig. 1A) is an allosteric inhibitor of CD45 phosphatase activity. It suppresses T-cell receptor signaling pathway in activated T cells but does not affect resting T cells, which express CD45 but do not require CD45 activity (Perron et al., 2014).

Treatment of EL4 cells ($CD45^+$) with compound 211 demonstrated an ~LD_{50} 0.5 ± 0.18 μM at 24 hours (Fig. 1B). The $CD45^+$ human B-cell lines Ly-1 and Toledo were also sensitive to compound 211. $CD45^+$ Jurkat T cells were sensitive to compound 211 whereas the $CD45^-$ Jurkat T cell variant J.1 were resistant to compound 211. Control $CD45^-$ cells human HEK293 fibroblasts and human KB nasopharyngeal tumor cells were not sensitive, further demonstrating that compound 211 sensitivity requires CD45 expression (Table 1).

CD45 Inhibition Hyperactivates Lck Leading to Cell Cycle Arrest and Apoptosis of EL4 Cells. Inappropriate T-cell activation results in a G2/M cell cycle arrest and cell

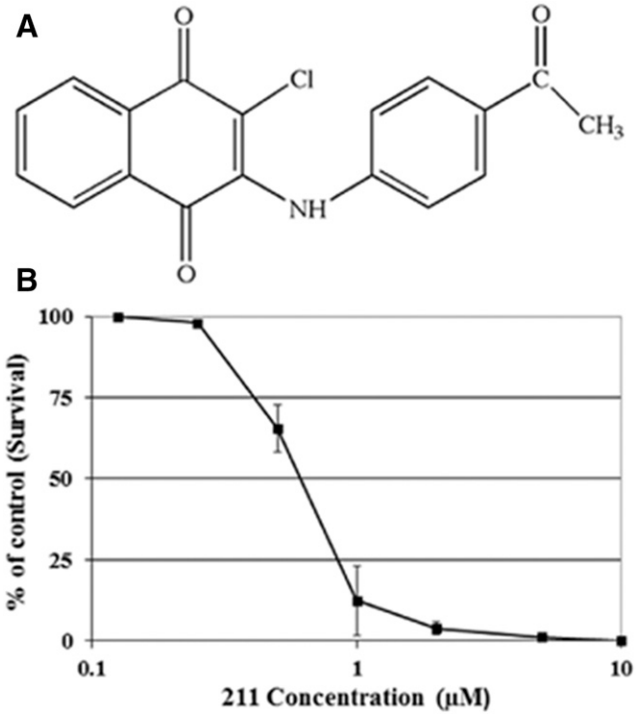

Fig. 1. CD45 inhibitor 211 reduces the viability of EL4 lymphoma cells in culture. (A) Compound 211, a selective small-molecule allosteric inhibitor of CD45 phosphatase activity. (B) Dose-response inhibition of EL4 cell viability after treatment with compound 211 for 24 hours. As described in Table 1, the $LD_{50} \pm$ S.D. for compound 211 is 0.5 ± 0.18 μM ($n = 6$ wells per assay, data averaged from three independent assays).

TABLE 1

Cell death induced by compound 211 treatment specific to CD45-expressing T and B cell lines

Cells were treated in culture media for 24 or 48 hours with a range of compound 211 concentrations or vehicle, and the LD_{50} (micromolar) were determined from growth curves (as in Fig. 1B). Average, $n = 6$ replicate wells per condition. For simplicity, S.D. is not shown; in all cases S.D. <0.48. All $CD45^+$ cells are sensitive to compound 211 ($LD_{50} < 2.6$ μM), whereas all $CD45^-$ are insensitive to compound 211 (undetectable LD_{50} at 10 μM).

LD_{50}	$CD45^+$ Cells					$CD45^-$ Cells		
	EL4	5C3	Jurkat	Ly-1	Toledo	Jurkat J1	KB	HEK293
At 24 hours	0.5	0.2	1.6	2.6	1.9	>10	>10	>10
At 48 hours	0.3	0.1	0.9	1.7	1.2	>10	>10	>10

death (Kishihara et al., 1993; Byth et al., 1996; Mee et al., 1999; Falahati and Leitenberg, 2008). Hence, we explored whether G2/M cell cycle arrest and cell death are mechanisms by which compound 211 compromises viability in $CD45^+$ cells.

Because the autocatalytic p-Tyr p-Lck Y394 is a substrate for CD45 dephosphorylation, the phosphorylated state of Y394 can be used as a surrogate marker of CD45 activity. EL4 cells were treated with 0.5 μM compound 211 or control vehicle for 3 hours, and total Lck was immunoprecipitated from detergent lysates. The Lck samples were Western blotted using antibodies to detect p-Lck Y394. Untreated EL4 cells growing in serum have high basal p-Lck Y394 levels. Treatment with compound 211 for 3 hours induced a statistically significant increase in p-Lck Y394 (Fig. 2A). Standardization of the data to total Lck loaded and quantification of $n = 3$ independent assays showed a statistically significant ($P < 0.01$) ~75% increase in p-Lck Y394 with respect to baseline (Fig. 2A), demonstrating that CD45 inhibition causes abnormal Lck hyperphosphorylation.

To study cell cycle events after abnormal Lck activation, EL4 cells were treated for 15 hours with 0.5 μM compound 211 or control vehicle, then were permeabilized and stained with propidium iodide (PI) to quantify DNA content and cell cycle progression by flow cytometry (Fig. 2B). In vehicle-treated control cells, the expected percentage of cells in G1 phase (50 ± 5), S phase (21 ± 7), and G2/M phase (29 ± 6) were detected. In cells treated with compound 211 the percentage of cells in G1 phase remained constant (49 ± 4), but the percentage of cells in S phase decreased (7 ± 6), and the percentage of cells in G2/M phase increased (44 ± 7). These data indicate that after completion of the S phase, cells arrest at G2/M and do not return to the G1 phase of the cell cycle.

The cells arrested in the G2/M cell cycle undergo cell death. In these studies, cells were treated with 0.5 μM compound 211 or control vehicle for 24 hours, and the levels of Annexin V-FITC antibody (apoptotic cells) and PI (dead/necrotic cells) were quantified by flow cytometry (Fig. 2C). Note that at 24 hours this dose is the LD_{50} of compound 211in metabolic/proliferation assays (Table 1). Quantification of flow cytometry data showed that after 24 hours of compound 211 treatment ~50% of the cells stain for both Annexin V and PI, indicating apoptosis/necrosis, and this is consistent with the data from metabolic/proliferation assays (Table 1). There was no apoptosis/necrosis after 3 or 15 hours of treatment with compound 211 compared with controls, indicating a correlation of a time-dependent progression through the cell cycle and death. In addition, death is CD45-dependent because cells lacking CD45 were not affected by compound 211 (Table 1), ruling out nonspecific toxicity.

Together, the data indicate that the inhibition of CD45 phosphatase enzymatic activity by compound 211causes abnormal phosphorylation of p-Lck Y394 within 3 hours, leading to cell cycle arrest at the G2/M checkpoint within 15 hours and culminating in late-stage apoptosis within 24 hours.

Inhibition of CD45 Phosphatase Delays EL4 Tumor Growth and Reduces Metastasis In Vivo. Next, we tested the concept of pharmacologic inhibition of CD45 in a therapeutic paradigm of lymphoma in vivo. A dose of 3 mg/kg was selected for tumor therapy studies as it does not cause statistically significant lymphopenia, neutropenia, or serious adverse side effects (Perron et al., 2014).

EL4 cells syngeneic to the C57/BL6 strain of mice are very aggressive and metastatic and grow in 100% of mice tested. Subcutaneous injection of EL4 cells in all mice results in palpable primary tumors after 5 days and in quantifiable primary tumors after 7 days. The tumors metastasize to lymph nodes, which quantifiably increase in size. Treatment was initiated at day 5 of tumor implantation (once the primary tumor was palpable), and primary tumor volumes were measured thereafter. Control vehicle or compound 211 were administered daily (3 mg/kg via intraperitoneal injection; $n = 7$ tumor-bearing mice per group) (Fig. 3A).

Treatment with compound 211 resulted in a statistically significant inhibition of the growth rate of the primary tumor from day 12 onward, compared with the vehicle-treated group. Overall there was a ~50% reduction in primary tumor size at day 14 and a ~40% reduction in tumor size at day 18 (Fig. 3B). These data were reproduced in three independent experiments.

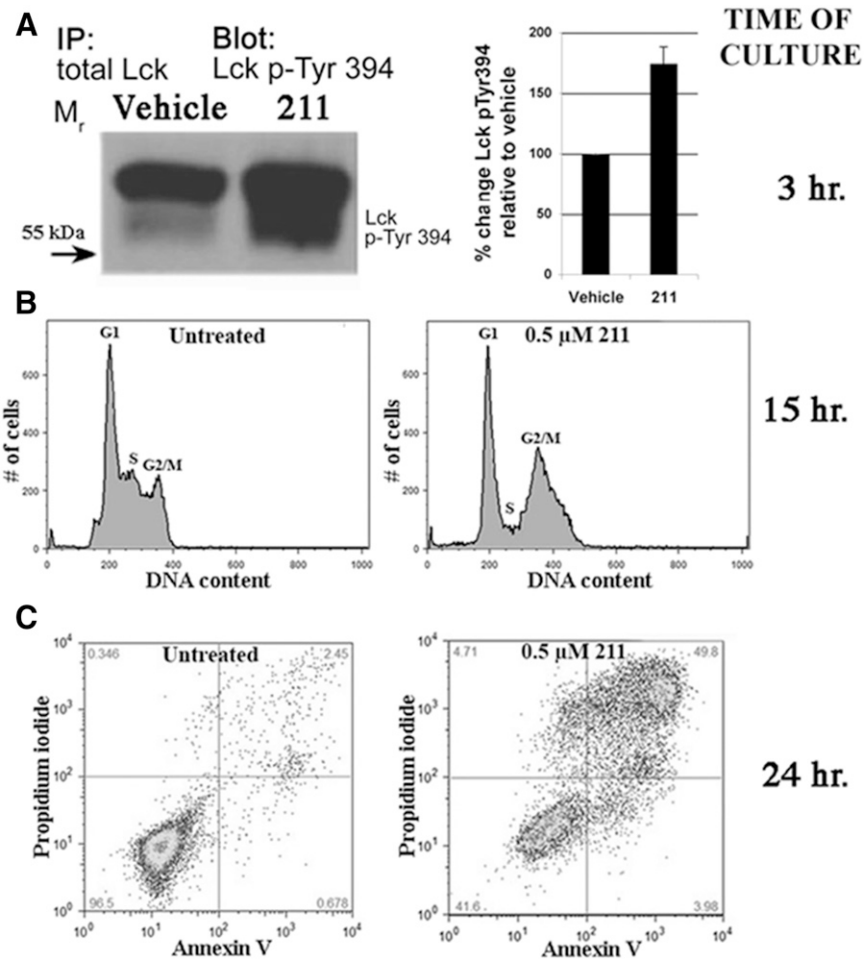

Fig. 2. Sequence of events leading to EL4 cell apoptosis after treatment with compound 211. All experiments were performed as $n = 3$ independent assays. (A) EL4 cells were treated with compound 211 or vehicle for 3 hours. Cell lysates were immunoprecipitated with anti-Lck antibody, and samples were immunoblotted with anti-p-Lck Y394 antibodies. The Western blot shown is representative, and the bar graph shows quantification of $n = 3$ Western blots using independent samples, normalized to total Lck and to vehicle treatment. (B) EL4 cells were treated with compound 211 or vehicle for 15 hours, followed by permeabilization and staining with PI to quantify DNA content (and cell cycle) by flow cytometry of 5000 cells. Compound 211 causes a cell cycle arrest at G2/M. The G1 peak remains unchanged while the S peak is reduced. The percentage of cells in each stage of the cycle are indicated in the text. (C) EL4 cells were treated with compound 211 or vehicle for 24 hours, and without permeabilization they were immunostained with annexin V-FITC antibody (apoptosis marker) and with PI (necrosis marker). The percentage of cells are indicated in each quadrant. Untreated cells in the lower-left quadrant indicate a live cell population. Treatment with compound 211 induces shifts of ~50% of cells into the upper-right quadrant, indicating late-stage apoptosis and necrosis in this population.

The mice were euthanized on day 18 after tumor implantation, and lymph node metastasis was quantified by measuring the axillary lymph nodes contralateral to the tumor implantation site. These lymph nodes are representative of therapeutic efficacy at the metastatic sites for two reasons. First, these lymph nodes were on the opposite side from the subcutaneous primary tumor, so tumor cells found in these lymph nodes were the consequence of metastasis. Second, these lymph nodes were not located in the peritoneal cavity where drug was given, so they were not exposed directly to the injected drug.

The vehicle-treated group had statistically significant lymph node enlargement due to the infiltration of EL4 tumor cells, which was verified by phenotyping for a tumor-specific marker (Supplemental Fig. 2, immunostained with anti-GD2 mAbs as per Tong et al., 2015). In tumor-bearing mice (compared with non-tumor-bearing mice) the size of the nodes in the vehicle-treated mice was increased ~12 times that of normal; in the compound 211-treated mice the size of the nodes was increased ~6 times that of normal (Fig. 3C). This represents a decrease of ~50% in metastatic mass. Representative examples of lymph nodes from each group are shown in Fig. 3D.

Inhibition of CD45 Phosphatase Delays 5C3 Tumor Growth In Vivo. We expanded the in vivo tests to a liquid tumor. We used the 5C3 hybridoma, a fusion of a B cell and an SP2/0 myeloma, producing a mAb. The 5C3 hybridoma grows in the peritoneum, causing an expanded abdomen visible after 4 days and ascitic fluid containing mAb in the peritoneal exudate. Treatment was initiated at day 4 with vehicle or compound 211 administered every other day (3 mg/kg via intraperitoneal injection; $n = 8$ tumor-bearing mice per group). The treatment frequency was reduced to every other day because the drug is given in the peritoneal cavity where the tumor cells grow, so the tumors are exposed directly to the drug. Treatment with compound 211 resulted in statistically significant differences versus the control group, with a reduction of hybridoma cells in the exudate and a decrease in mAb titer as measured at the day 11 end point (Table 2).

Discussion

We have validated in vivo targeting of CD45 enzymatic activity rather than CD45 protein as a potential therapeutic strategy for bone marrow–derived cancers. Neoplasias of the immune system, including rapidly proliferating lymphomas and leukemias, ubiquitously express CD45, and CD45 activity plays a critical role for their proliferation and survival by regulating SFKs such as Lck, Fyn, and Lyn. We hence postulated that phosphatase activity can be a target for inhibition of cancer cell growth. This approach would spare normal nonproliferating cells that also express CD45 but do not depend on CD45 activity.

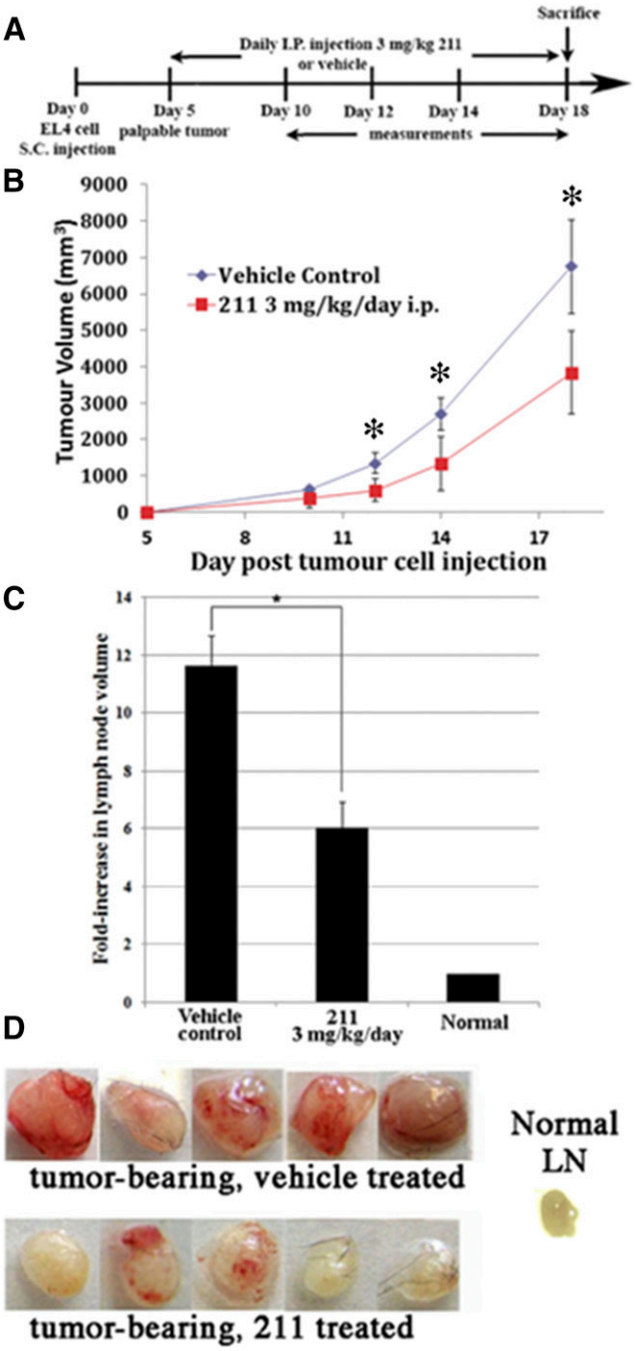

Fig. 3. Inhibition of CD45 reduces EL4 tumor growth rate and metastasis in vivo. L4 cells are syngeneic to C57Bl/6, and were used in tumor studies. (A) On day 0, 2×10^5 EL4 cells were injected on the flank of mice; the tumor was palpable by day 5. At this point mice were divided into two groups (n = 7 per group) that received daily intraperitoneal injections of either 3 mg/kg compound 211 or vehicle. The primary tumor size was measured on days 10, 12, 14, and 18, before euthanasia. (B) Average tumor size ± S.D. at the times indicated. Beginning at day 12, there is a statistically significant difference in tumor volume between the groups treated with compound 211 and vehicle (*$P < 0.05$). The data are representative of three independent experiments. See Supplemental Fig. 1 for two additional experiments. (C) After the mice were killed, the contralateral lymph nodes (n = 7 per group) were removed and their weight quantified. Lymph nodes in the vehicle-treated group are larger, indicating a higher rate of metastasis in these animals. (D) Examples of lymph nodes, tumor-bearing ± drug treatment, and naïve mouse not bearing a tumor.

TABLE 2

Inhibition of CD45 reduces 5C3 ascites growth and mAb production in vivo

5C3 hybridoma cells, which are syngeneic to Balbc/C mice, were used in tumor studies. On day 0, 1×10^6 5C3 tumor cells were injected intraperitoneally, and they grew and generated ascitic fluid. At day 4 mice (n = 8 per group) received intraperitoneal injections of either 3 mg/kg compound 211 or vehicle at days 4, 6, 8, and 10. At day 11 all peritoneal fluid was collected; the cell numbers were counted and mAb titers quantified by serial dilution (a high dilution indicates a high titer of mAb). Average ± S.D., n = 8.

	Compound 211	Vehicle
Ascites, volume (ml)	0.6 ± 0.3[a]	2.3 ± 0.4
Ascites, cells/ml ($\times 10^6$)	15.8 ± 4.7[a]	33.7 ± 8.9
Average cell number ($\times 10^6$)	9.5 ± 6.2[a]	77.5 ± 20.4
Binding saturation (dilution)	1:625	1:10,000

[a]$P < 0.01$, comparing compound 211 treatment with vehicle.

Compound 211 is a small-molecule selective inhibitor of CD45, with an allosteric mechanism of inhibition that was effective at preventing inflammation in vivo (Perron et al., 2014). Here we show that inhibition of CD45 phosphatase activity by compound 211 resulted in the death of tumor cell lines in vitro in a dose-dependent and CD45-dependent manner. The mechanism of cell death involves dysregulation of Lck and inappropriate activation, leading to cell cycle arrest and ending in apoptosis/necrosis. Other studies have reported that dysregulation of CD45 using mAbs or soluble CD45 enzyme can result in cell death or lack of differentiation/growth (Robson et al., 1996; Harashima et al., 2002; Puck et al., 2017). However, to the best of our knowledge, our study is the first to associate inhibition of CD45 activity and cell cycle arrest, a mechanism that leads to cell death.

We studied therapeutic paradigms for a T-cell and a B-cell cancer, but it may be possible to target CD45 in other lymphoid cancers such as multiple myeloma and in gliomas, and it may be useful in preventing transplant rejection or autoimmunity, and other pathologies.

Antibodies directed to the extracellular domain of CD45 have been used for cancer management (Hamaguchi et al., 2001; Nemecek and Matthews, 2002; Pagel et al., 2003). One problem with the use of anti-CD45 mAbs is that these agents target all cells expressing CD45 (normal resting and rapidly proliferating lymphoid cells), regardless of whether CD45 is functionally essential to that cell. Systemic depletion of CD45 compromises the body's ability to mount a desirable immune response to infections; individuals who lack expression of CD45 exhibit a severe combined immunodeficiency phenotype (Tchilian et al., 2001). Indeed, the in vivo pharmacologically effective dose of compound 211 did not cause detectable toxicity or abnormalities in the normal immune repertoire (see the supplemental material in Perron et al., 2014).

Sparing normal resting lymphoid cells—which *do not* require CD45 activity—while targeting rapidly proliferating lymphoid cells—which *do* require CD45 activity—(McNeill et al., 2007) would be beneficial. A rapidly proliferating lymphoid cell that is a cancer cell would dysregulate the SFK signals that are validated targets for cancer therapy (Zhao et al., 2008; Harr et al., 2010).

Regarding normal resting cells, once the compound 211 inhibitor is eliminated or cleared from the system, the surviving normal resting T cells should resynthesize new CD45 protein; enzymatically active, they would be able to mount a normal immune response when necessary. This view is supported by

our data showing that normal resting lymphoid cells cultured ex vivo do not die when exposed to the inhibitor compound 211 (Perron et al., 2014). On the other hand, the normal lymphoid cells that attempt to activate during therapy with CD45 inhibitor are susceptible to the drug and will probably die.

In the tumor therapy experiments reported here, we treated mice with compound 211 monotherapy, which proved effective in slowing primary tumor growth and reducing lymph node metastasis. However, the CD45 inhibitor compound 211 did not completely eradicate the tumor, so in future we will test compound 211 as an adjuvant to other forms of therapy.

Acknowledgments

The authors thank Dr. Koren Mann for cells and reagents, Dr. Wenyong Tong and Yina Xu for help with animal experiments, Taraneh Taghavi for help with MTT assays, and Dr. Fouad Brahimi for help with flow cytometry assays.

Authorship Contributions

Participated in research design: Perron, Saragovi.

Conducted experiments: Perron, Saragovi.

Performed data analysis: Perron, Saragovi.

Wrote or contributed to the writing of the manuscript: Perron, Saragovi.

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Address correspondence to: Dr. H. Uri Saragovi, McGill University, Lady Davis Institute-Jewish General Hospital, 3755 Cote St. Catherine., E-535, Montreal, QC H3T 1E2, Canada. E-mail: Uri.Saragovi@mcgill.ca