A novel orally available inhibitor of focal adhesion signaling increases survival in a xenograft model of diffuse large B-cell lymphoma with central nervous system involvement

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ABSTRACT

Central nervous system dissemination is a relatively uncommon but almost always fatal complication in diffuse large B-cell lymphoma patients. Optimal therapy for central nervous involvement in this malignancy has not been established. In this paper, we aimed to evaluate the therapeutic effect of E7123, a celecoxib derivative that inhibits focal adhesion signaling, in a novel xenograft model of diffuse large B-cell lymphoma with central nervous system involvement. Cells obtained after disaggregation of HT subcutaneous tumors (HT-SC cells) were intravenously injected in NOD/SCID mice. These mice received oral vehicle or 75 mg/kg of E7123 daily until they were euthanized for weight loss or signs of sickness. The antitumor effect of E7123 was validated in an independent experiment using a bioluminescent mouse model. Intravenously injected HT-SC cells showed higher take rate and higher central nervous system tropism (associated with increased expression of β1-integrin and p130Cas proteins) than HT cells. The oral administration of E7123 significantly increased survival time in 2 independent experiments using mice injected with unmodified or bioluminescent HT-SC cells. We have developed a new xenograft model of diffuse large B-cell lymphoma with central nervous system involvement that can be used in the pre-clinical evaluation of new drugs for this malignancy. E7123 is a new, well-tolerated and orally available therapeutic agent that merits further investigation since it may improve current management of diffuse large B-cell lymphoma patients with central nervous system involvement.

Introduction

Central nervous system (CNS) dissemination has been reported in 2-10% of diffuse large B-cell lymphoma (DLBCL) patients.[1,2] Although relatively uncommon, it is fatal in over 90% of patients.[3,4] Most CNS metastases occur in the setting of DLBCL relapse and their treatment is mostly palliative.[5] The factors most consistently associated with risk of CNS recurrence are elevated serum lactate dehydrogenase and involvement of more than one extranodal site.[6-9] CNS-directed prophylactic intrathecal therapy is often used to reduce the risk of CNS recurrence in these patients but its benefit has been questioned in recent years.[1,10,11] The addition of rituximab to standard cyclophosphamide, doxorubicin, vincristin and prednisone (CHOP), has improved the outcome of DLBCL patients,[12-16] but the protective effect of rituximab on CNS recurrence is controversial.[2,17-21] Thus, the optimal treatment for CNS dissemination in DLBCL has not yet been established and new therapies are needed in order to prevent or treat this fatal condition. However, the lack of appropriate animal models that reproduce CNS involvement in DLBCL hinders the development of new drugs.

Brain metastasis is a multistep process of tumor cell attachment to microvessel endothelial cells, extravasation into the brain, interaction with the local microenvironment and proliferation. This process involves precise regulation of cell-cell adhesion and cell-extracellular matrix (ECM) adhesion.[22,23] The cell-ECM adhesion is maintained by the focal adhesion complexes, composed of transmembrane receptors known as integrins, structural proteins (i.e. vinculin, talin, paxilin) and signaling proteins (i.e. FAK, p130Cas, HEF1, Pyk2).[24] A critical step in the CNS metastasis process is tumor cell adhesion to the vascular basement membrane of the brain which is mediated by integrin signaling.[25,26] Thus, agents that efficiently block tumor cell adhesion mediated by focal adhesion signaling in DLBCL may prevent CNS progression.

We recently found that E7123, a novel non-COX-2 celecoxib derivative capable of inhibiting focal adhesion signaling, had *in vitro* antitumor effect against DLBCL cell lines and reduced tumor volume in a NOD/SCID subcutaneous model of DLBCL.[27] Our group has also shown that subcutaneous conditioning of cells prior to intravenous injection of DLBCL cell lines increased tumor take rate and cell dissemination capacity as compared to direct intravenous injection in NOD/SCID mice.[28] In this paper, we aimed to evaluate the therapeutic effect of E7123 in a novel xenograft mouse model

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of DLBCL with CNS involvement. The animal model was generated by performing a subcutaneous passage of HT cells, which had previously shown CNS tropism after direct intravenous injection, before their intravenous injection in NOD/SCID mice.

Design and Methods

Cell line and compounds

HT human DLBCL cell line (DMSZ Cell Line Bank) was cultured in RPMI 1640 supplemented with 10% fetal bovine serum, 1% glutamine, 100 units/mL penicillin/streptomycin (Life Technologies) and incubated at 37°C in a humidified atmosphere containing 5% CO_2. HT-SC cells or bioluminescent HT-Luc-SC cells were obtained from HT or HT-Luc subcutaneous tumors, respectively (*see below*). E7123 was synthesized by the HSCSP Pharmacy Department and Laboratories Esteve S.A.

Lentiviral infection of HT cells

A plasmid containing the Luciferase gene (Luc) was constructed by subcloning and replacing the GFP1 gene of the plasmid pSIN-DUAL-GFP1-GFP2, kindly provided by Dr Mary Collins (College Medical School, Cleveland, OH, USA). Luciferase cDNA was obtained from the plasmid pPK-CMV-F3 (C-Luc) (Promokine, Heidelberg, Germany). Lentiviral particles containing the pSIN-DUAL-Luciferase-GFP2 vector, were used to infect HT cells as described previously by our group.[27] Infected cells, termed HT-Luc cells, were used for *in vivo* experiments.

Animal experiments

Animal procedures were approved by the Hospital Sant Pau Animal Ethics Committee according to established guidelines. NOD/SCID mice were subcutaneously inoculated with HT or HT-Luc cells. Cells obtained from subcutaneous tumors, HT-SC or HT-Luc-SC, were intravenously injected in mice to evaluate the aggressiveness of the subcutaneous conditioning and to evaluate E7123 antitumor effect. Mice were orally administered with E7123 (75 mg/kg) or vehicle (PEG:FBS) until they were euthanized (*Online Supplementary Figures S1 and S2*). End points were a 15% loss in body weight or the appearance of motor problems or evident signs of sickness. *In vivo* bioluminescence imaging (BLI) was performed as previously described.[29] Dorsal BLI pictures were taken once a week (ORCA-II Deep Cooling BTW Imaging System, Hamamatsu Photonics, Hamamatsu City, Japan) and quantified as described in the *Online Supplementary Appendix*.

Immunohistochemistry

Paraffin-embedded tissue sections of HT-SC or HT infiltrated brains were used to detect CD20, CD10, Ki67 (DAKO, Carpinteria, CA, USA), p130Cas (Neomarkers, Fremont, CA, USA) and β1-integrin (Upstate, Billerica, MA, USA) proteins. Immunohistochemical reactions were performed in a DAKO Autostainer Link48 following the manufacturer's instructions. All slides were viewed with an Olympus BX51 microscope. Images were acquired using an Olympus DP72 digital camera and processed with the Olympus Cell^D Imaging 3.3 software (Olympus Corporation, Tokyo, Japan).

Western blot analysis

HT-SC cell lysates were used to evaluate protein expression. Western blots were performed as previously described.[30] The following primary anti-human antibodies diluted in TBS-T, containing 0.1% BSA, were used: mouse anti-p130Cas, anti-AKT, anti-FAK, anti-FAK Tyr397, anti-Pyk2, anti-Lyn, anti-Mcl1, anti-integrinβ1 (BD Pharmingen, San Jose, CA, USA), rabbit anti-AKT Thr308 and anti-Src (Cell Signalling, Danvers, MA, USA) and goat anti-β-actin (Santa Cruz Biothecnology, CA, USA). The secondary antibodies used were anti-mouse-IgG and anti-rabbit IgG (Jackson Immunoresearch, West Grove, PA, USA).

Statistical analysis

Differences in survival between control HT or HT-SC groups and between HT-SC or HT-Luc-SC mice receiving vehicle or drug were calculated using Kaplan-Meier curves and the log rank test. Fisher's exact test was used to calculate differences between categorical variables. $P<0.05$ was considered statistically significant. Further details are described in the *Online Supplementary Appendix*.

Results

Subcutaneous passage of the HT cell line enhanced engraftment

Previous studies revealed that direct intravenous injection of DLBCL cells in NOD/SCID mice showed inefficient take rate and took a long time to generate lymphoma. However, when we performed a subcutaneous passage of the same DLBCL cell lines before their intravenous injection in mice their aggressiveness was dramatically increased, producing a disseminated lymphoma in all mice in a short period of time.[28] In a preliminary experiment, we observed that direct intravenous injection of HT cells produced lymphoma in 2 of 10 mice at 66 and 74 days after injection, and that both mice showed CNS infiltration. In order to increase the take rate and the aggressiveness of these cells, we performed a subcutaneous passage of HT cells before their intravenous injection, and compared their take rate and the animal survival with control mice directly injected with HT cells, without previous subcutaneous passage (see *Design and Methods*). Cells obtained after disaggregation of HT subcutaneous tumors were named HT-SC cells. In the intravenously injected HT-SC group, one mouse had to be excluded from the experiment as a tumor developed in the injection point. The remaining mice in this group (n=7) were euthanized between Days 40 and 51 post-injection, when they reached a 15% weight loss. In contrast, only 2 of 10 HT control mice were euthanized at Days 78 and 89 after injection as a consequence of weight loss. The remaining mice in the HT control group were sacrificed at Day 100 without any sign of disease and histopathological analysis revealed no tumor development. There was a statistically significant difference in survival times between groups ($P<0.01$) as shown by Kaplan-Meier analysis (Figure 1A). Interestingly, mice from the experimental HT-SC group showed a visible cranial inflammation at approximately Day 30 after injection.

Intravenous injection of HT-SC cells generated DLBCL in the CNS

Most mice showed motor difficulties (generally circular motion) before being sacrificed. At necropsy, we observed that mice from the experimental group showed an evident cranial inflammation in comparison with mice from the HT control group (Figure 1B). When analyzed microscopically, we observed that 100% of HT-SC mice showed brain infiltration, especially in leptomeninges (Figure 2A-H), whereas only one of 2 infiltrated HT control mice showed this involvement (Table 1). Infiltrated brains of the HT-SC mice were positive for CD20 (B-cell antigen) (Figure 2I) and CD10 (germinal center marker) (Figure 2J)

immunostaining, confirming that HT-SC cells maintained the germinal center DLBCL phenotype found in the original HT cells. Immunostaining with the proliferation marker Ki67 (Figure 2K) showed a proliferation index of 70-80% in all lymph nodes, a common proliferation rate in DLBCL pathology

HT-SC cells showed higher p130Cas and β1-integrin expression than HT cells

Based on previous evidence showing that *in vivo* passages were associated with changes in adhesion properties,[28,31-33] and that the expression of some adhesion molecules could be associated with CNS-tropism,[23,25] we studied the expression of β1-integrin, p130Cas, FAK-P, Pyk2, Lyn and Src adhesion proteins in HT cell lysates before (control HT) and after (HT-SC) the subcutaneous passage. We also assessed other proteins associated with the focal adhesion pathway, such as Akt-P, Akt and Mcl-1.[27] We detected a marked increase in p130Cas and β1-integrin expression levels and a slight increase in Src and Mcl-1 expression levels in HT-SC cells as compared to HT cells. However, there was no alteration in FAK, FAK-P, Akt, Akt-P, Pyk2 and Lyn proteins during the subcutaneous conditioning of cells (Figure 3A). Immunohistochemistry of β1-integrin and p130Cas was performed in HT cells before the subcutaneous passage (HT control), in the subcutaneous tumors, in the cells obtained after tumor disaggregation (HT-SC), and in brain tissue sections (Figure 3B). The intensity of the β1-integrin and p130Cas immunostaining was clearly higher in subcutaneous tumors and in HT-SC cells than in the control HT cells. We also observed that this high intensity was retained in HT-SC cells at the brain metastases generated after their intravenous injection in mice. β1-integrin and p130Cas staining was always localized in the cytoplasm and membrane in HT cells, subcutaneous tumors, HT-SC cells and infiltrated brains.

E7123 increased survival time in the DLBCL animal model with CNS involvement

Ten days after intravenous injection of HT-SC cells, mice were randomly divided into two groups and treated orally with 75 mg/kg E7123 or vehicle. Mice were treated every day until they were sacrificed for weight loss or signs of sickness. We used PEG-400:FBS 3:1 as a vehicle because it showed no toxicity in healthy mice and permitted the total solubilization of E7123 without changing its antitumor activity *in vitro*.[27] All mice receiving vehicle were sacrificed between Days 39 and 48 post-injection. However, mice receiving E7123 were sacrificed between 43 and 85 days after injection. There was a statistically significant difference in survival time between both groups (P=0.01) as assessed by the log rank test (Table 2). Table 2 also shows the microscopic infiltration of brain, lymph nodes or bone marrow of all mice.

E7123 increased survival time in a validation experiment using a bioluminescent mouse model of DLBCL with CNS infiltration

In order to validate the antitumor effect of E7123 in the new xenograft model of DLBCL disseminated to the CNS, we performed the same procedure using bioluminescent subcutaneously-conditioned HT cells (HT-Luc-SC).

HT-Luc-SC cells were intravenously injected in 20 NOD/SCID mice that were randomly divided into two groups receiving vehicle or 75 mg/kg E7123. The first CNS bioluminescent signal was observed in 3 vehicle-treated and 2 E7123-treated mice on Day 17 post-injection. CNS bioluminescence was observed in all mice between Days 17 and 57 after cell injection. Figure 4A shows the time course BLI signal in CNS of representative vehicle and E7123-treated mice. We could observe that the BLI signal in the CNS of the vehicle-treated mouse increased faster than in the E7123-treated mouse. BLI intensity quantification curves for both groups are represented in Figure 4B. Thus, DLBCL cell growth in CNS was delayed in E7123-treated mice. However, E7123 did not block lymphoma cell invasion of the CNS since there were no significant differences in the time of initial detection of CNS BLI signal between groups.

E7123 also increased mice survival time in this model since vehicle-treated mice were killed between 36 and 58 days post-injection, whereas E7123-treated mice were euthanized between 48 and 91 days post-injection (Figure 4C). Two mice from each group were excluded from the

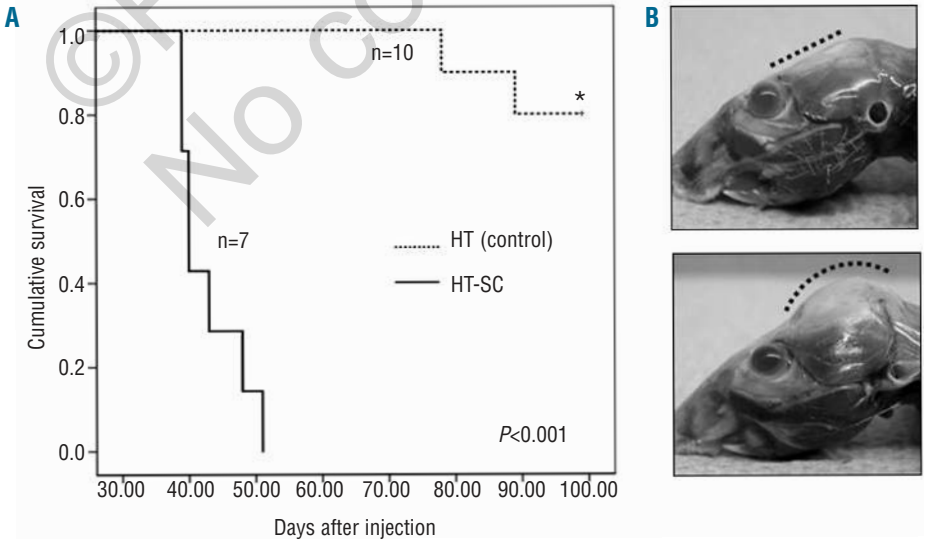

Figure 1. Survival in HT-SC mice is significantly lower than in control HT mice. (A) Kaplan-Meier analysis shows a significant reduction in survival time in HT-SC (n=7) as compared to control HT mice (n=10). *P* value for the log rank test was <0.01. (B) Macroscopic evidence for central nervous system involvement of DLBCL in NOD/SCID mice injected with HT-SC cells via the tail vein. *The remaining mice (n=8) were euthanized 95 days after injection without any sign of disease.

experiment because they did not develop lymphoma. Details of the histopathological features and clinical evolution of HT-Luc-SC injected mice are shown in Table 3.

Therefore, we confirmed that E7123 increased mouse survival time in a bioluminescent model of DLBCL mice with CNS infiltration. In addition, as observed in the luminescent images, E7123 slowed down the growth of lymphoma cells within the CNS.

Discussion

In this study, we showed that the oral administration of a novel focal adhesion inhibitor, E7123, increased survival of mice with DLBCL involving the CNS. The subcutaneous passage of HT cells before their intravenous injection in NOD/SCID mice allowed us to generate a xenograft model of DLBCL with CNS involvement that can be used for evaluation of drugs for this malignancy. We observed that, during the subcutaneous passage, HT cells acquired an increased expression level of β1-integrin and p130Cas proteins. This increased expression was associated with enhanced aggressiveness and CNS tropism of lymphoma cells.

The outcome of DLBCL patients with CNS occurrence is extremely poor and they do not appear to benefit from current prophylactic protocols.[2,9-11] Thus, there is a need to develop new treatments for this fatal condition. However, the development of new therapies against this malignancy is hindered by the lack of appropriate animal models to evaluate them. Some authors reported xenograft models of primary CNS lymphoma (PCNSL) performed after injection of the aggressive Burkitt's lymphoma cells directly into the brain,[34-37] whereas others generated syngeneic models of CNS lymphoma after intraocular or intracerebral injection of murine lymphoma cells in immunocompetent hosts.[38,39] An important advantage of the latter models in respect to xenograft models of CNS lymphoma using immunodeficient mice is that syngeneic models

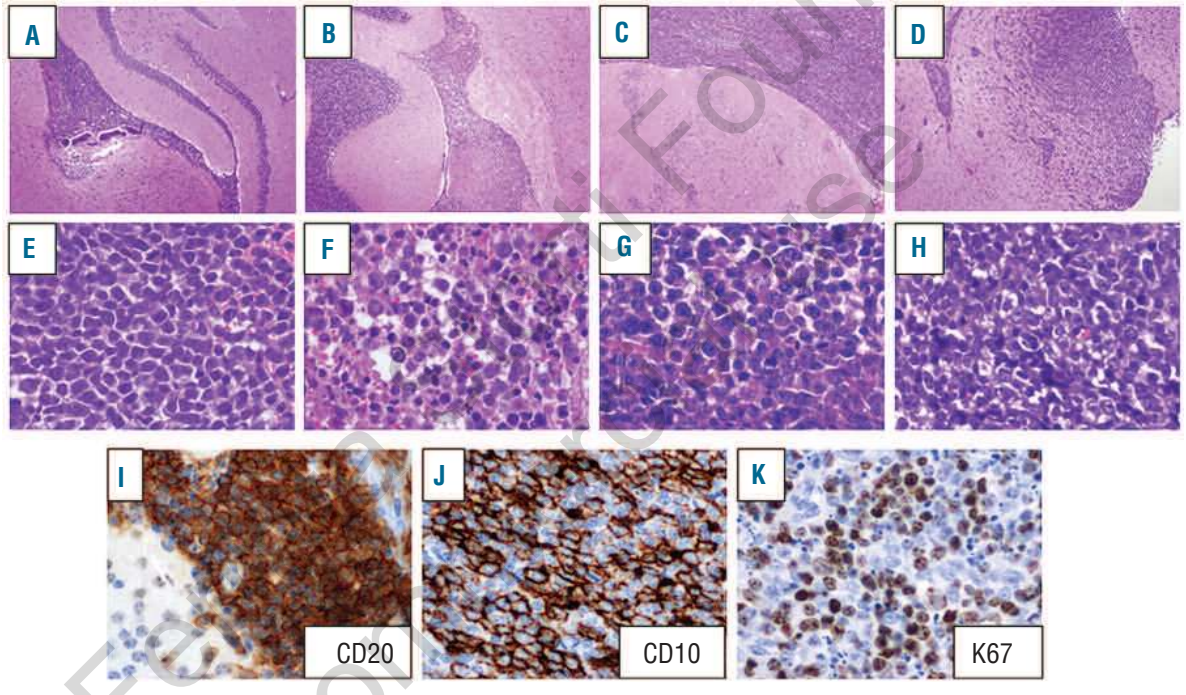

Figure 2. Histological and immunohistochemical phenotype of the DLBCL model with CNS involvement. H&E staining of representative brains in HT-SC mice. Lymphoma cells infiltrated the leptomeninges in most mice (A-D), reaching the parenchyma in isolated cases (D). Original magnification x40 and corresponding magnification x400 (E-H). Immunohistochemical analyses of CD20 (I), CD10 (J) and Ki67 (K) in representative sections of HT-SC infiltrated brains. Original magnification x400. Slides were viewed with an Olympus BX51 microscope. Images were acquired using an Olympus DP72 digital camera and processed with the Olympus Cell^{D} Imaging 3.3 software.

Table 1. Histopathological features and clinical evolution of NOD/SCID mice after intravenous injection of HT or HT-SC cells.

Clinical features	Control HT	HT-SC	*P*
N. of positive mice, n. (%)	2/10 (20)	7/7 (100)	0.002
Survival time (days)[1]	83.5±7.8	42.9±4.8	<0.001
N. of positive mice with CNS infiltration, n. (%)	1/2 (50)	7/7 (100)	0.222
Meningeal infiltration, n. (%)	1/2 (50)	7/7 (100)	0.222
Parenchymal infiltration, n. (%)	1/2 (50)	4/7 (57)	1.000
N. of positive mice with LN infiltration, n. (%)	0/2 (0)	1/7 (14)	1.000
N. of positive mice with BM infiltration, n. (%)	1/2 (50)	2/7 (29)	1.000

Positive mice are those mice that developed lymphoma. P values were calculated using the log rank test for survival time and Fisher's exact test for categorical variables. CNS: central nervous system; LN: lymph node; BM: bone marrow. [1]Values represent the mean ± SD.

allow the study of tumor-induced immunity.[40,41] However, they are technically difficult to perform and they do not use human DLBCL cells. Here, we generated a rapid xenograft model of DLBCL with CNS involvement achieved after a simple intravenous injection of HT-SC cells in NOD/SCID mice. Cells maintained the typical morphology of DLBCL cells and retained the germinal center DLBCL marker expression of the original HT cells. Our model also showed a 100% of meningeal infiltration, the most typical infiltrating pattern found in DLBCL patients with CNS involvement.[5] Moreover, the new bioluminescent model of DLBCL with CNS infiltration complements currently available murine models since it allows the study of the mechanisms of entrance, migration and growth of DLBCL cells in the CNS and their inhibition by drugs.

HT-SC cells used to generate this xenograft model showed higher levels of β1-integrin and p130Cas expression than HT cells. It has recently been reported that β1-integrin interaction with brain vessels is sufficient to promote proliferation and metastasis within the brain in carcinomas and lymphomas.[25,26,42] Moreover, p130Cas overexpression is associated with metastases in different cancer

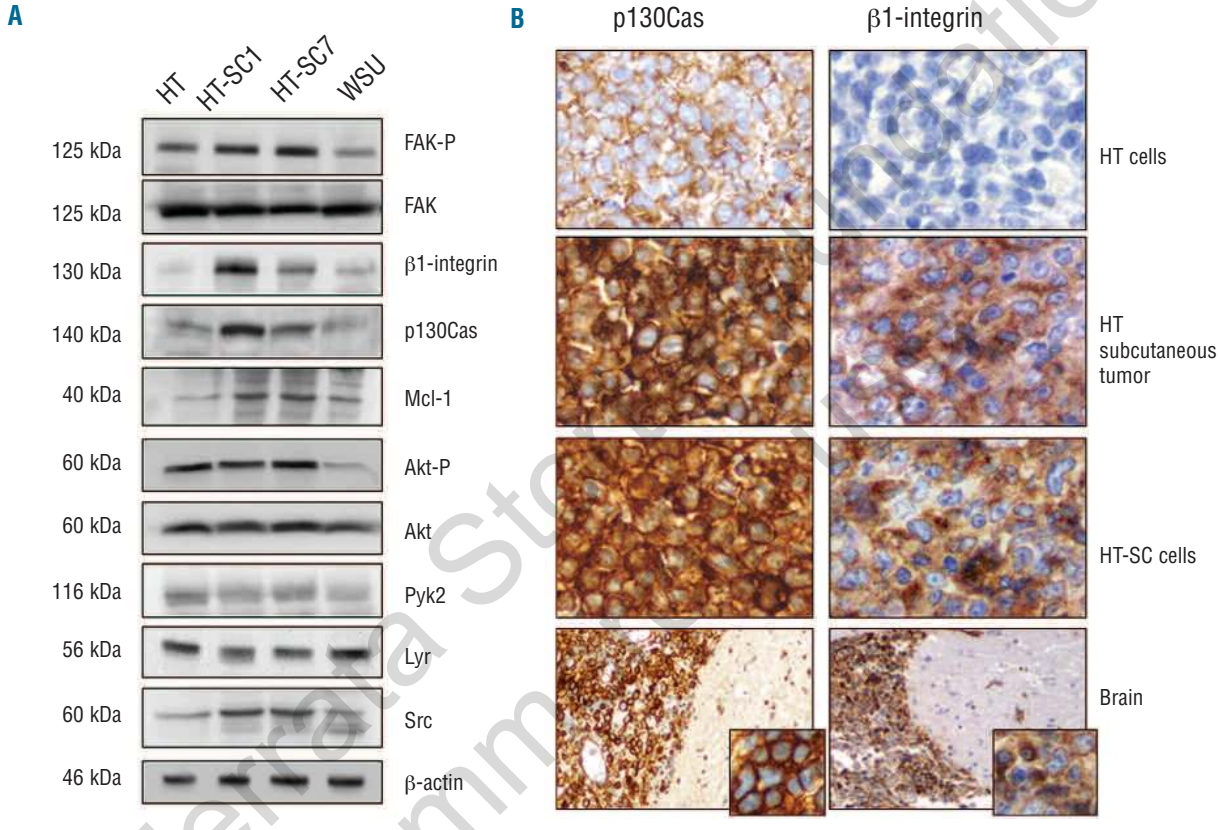

Figure 3. Expression of p130Cas and β1-integrin in HT and HT-SC cells. (A) Immunoblotting of FAK (Tyr 397), FAK, β1-integrin, p130Cas, Pyk2, Lyn, AKT (Thr 308) and AKT in protein extracts of control HT or HT-SC cells one day (HT-SC1) or seven days (HT-SC7) after disaggregation of subcutaneous tumors. WSU cell line lysate was used as positive control. HT-SC protein extracts showed higher expression levels of β1-integrin and p130cas than HT control cells. Equal loading was checked by immunobotting with β-actin. (B) Immunostaining analysis of p130Cas and β1-integrin in control HT cells, HT subcutaneous tumor, HT-SC cells (original magnification x400) and HT-SC infiltrated brain sections (original magnification x100, inset x400). Slides were viewed with an Olympus BX51 microscope. Images were acquired using an Olympus DP72 digital camera and processed with the Olympus Cell[D] Imaging 3.3 software.

Table 2. Histopathological features and clinical evolution of HT-SC injected NOD/SCID mice after administration of vehicle or E7123.

Clinical features	Vehicle	E7123	*P*
N. of positive mice, n. (%)	7/7 (100)	9/9 (100)	–
Survival time (days)[1]	42.9±4.8	61.1±15.4	0.001
N. of positive mice with CNS infiltration, n. (%)	7/7 (100)	9/9 (100)	–
Meningeal infiltration, n. (%)	7/7 (100)	9/9 (100)	–
Parenchymal infiltration, n. (%)	4/7 (57)	7/9 (78)	0.596
N. of positive mice with LN infiltration, n. (%)	1/7 (14)	2/9 (22)	1.000
N. of positive mice with BM infiltration, n. (%)	2/7 (29)	2/9 (22)	1.000

Positive mice are those that developed lymphoma. P values were calculated using the log rank test for survival time and Fisher's exact test for categorical variables. CNS: central nervous system; LN: lymph node; BM: bone marrow. [1]Values represent the mean ± SD.

types.[43,44] β1-integrin and p130Cas are components of the same signaling pathway which is associated with tumor invasion.[45] Thus, the high β1-integrin and p130Cas expression levels observed in HT-SC cells could partly explain the higher aggressiveness and capacity to migrate and grow into the CNS of DLBCL lymphoma cells as compared to HT cells. However, further functional studies are needed to determine whether β1-integrin and/or p130Cas are responsible for HT-SC cells tropism to the CNS and/or growth into the brain. In a previous study, the non-COX2 inhibitor celecoxib derivative E7123 showed an *in vitro* antitumor effect in different DLBCL cell lines by deregulation of focal adhesion proteins, which were expressed in the majority of human DLBCL patient samples. The oral administration of E7123 was well tolerated and reduced tumor growth in a subcutaneous xenograft of DLBCL.[27] In this paper, we showed that the focal adhesion inhibitor E7123 increases mice survival time in 2 independent experiments using a new xenograft model of DLBCL with CNS involvement. BLI images confirmed that E7123 did not block the CNS invasion, but delayed the growth of the DLBCL cells once in the brain, suggesting that E7123 may be capable of

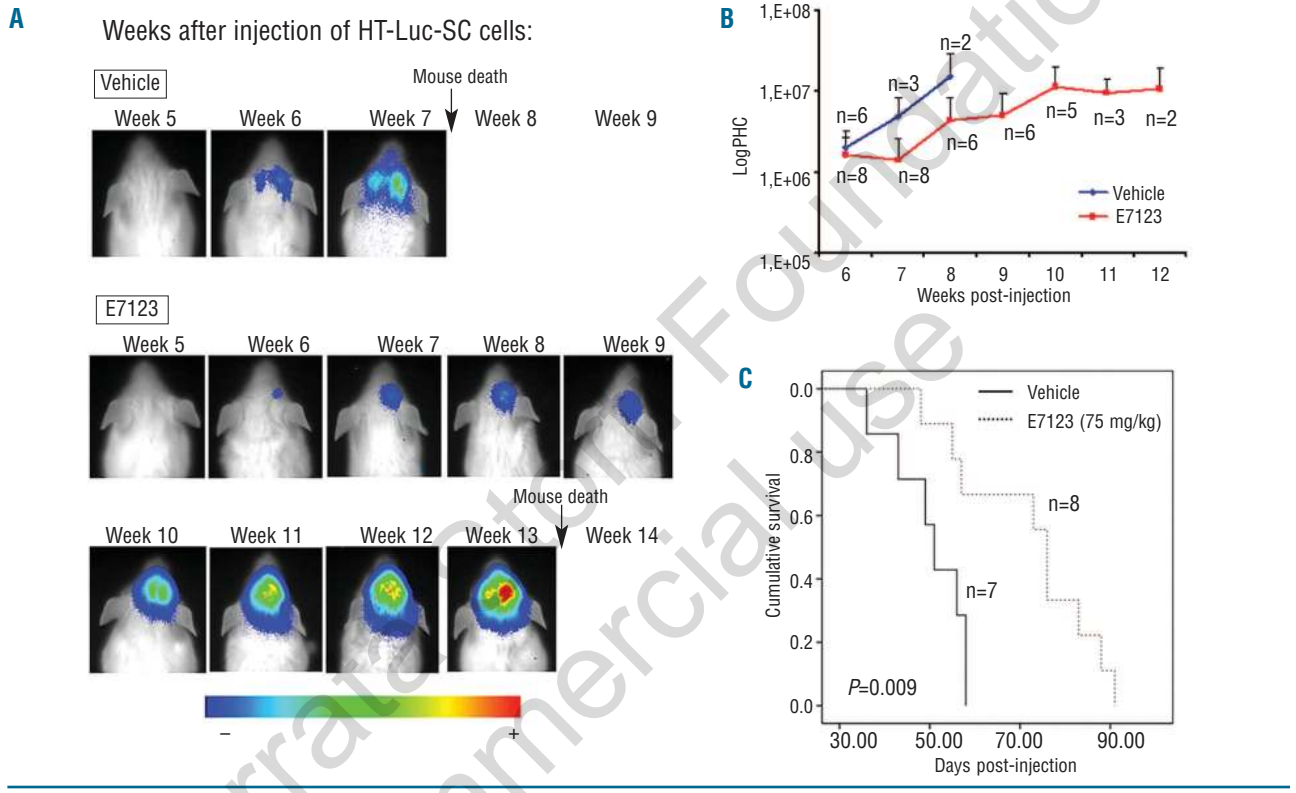

Figure 4. E7123 antitumor effect in an independent experiment using a bioluminescent model of DLBCL with CNS involvement. (A) Representative BLI images showing lymphoma HT-Luc-Sc cell growth evolution in the CNS in vehicle (PEG:FBS) or treated groups (75 mg/kg E7123). Vehicle-treated and E7123-treated mice were sacrificed on Days 56 and 92 post-injection of cells, respectively. Arbitrary color bars illustrate relative light intensity levels from firefly luciferase, ranging from low (blue) to high (red). Corresponding grayscale photographs and bioluminescent images were superimposed for each animal. (B) BLI signal quantification curves in the CNS for vehicle and E7123-treated mice. At week 6 after tumor cell injection, one vehicle-treated mouse could not be evaluated for BLI because it showed signs of sickness that precluded the use of anesthesia. BLI signal was quantified and expressed as Photon Counts (PHC). Curves represent the kinetics of BLI intensity mean for each group. Error bars represent the standard error. (C) Survival time in HT-Luc-SC mice treated with E7123 (75 mg/kg) was significantly higher than in control mice (PEG:FBS). *P* value for the log rank test was <0.01. Kaplan-Meier analysis showed the significant increase in survival time in treated mice (n=8) as compared to control mice (n=7).

Table 3. Histopathological features and clinical evolution of HT-Luc-SC injected NOD/SCID mice after administration of vehicle or E7123.

Clinical features	Vehicle	E7123	*P*
N. of positive mice, n. (%)	8/10 (80)	8/10 (80)	–
N. of positive mice with CNS infiltration, n. (%)	7/8 (87.5)	8/8 (100)	1.000
Meningeal infiltration, n. (%)	7/7 (100)	8/8 (100)	–
Parenchymal infiltration, n. (%)	5/7 (71.4)	7/8 (87.5)	0.596
N. of positive mice with LN infiltration, n. (%)	0/8 (0)	2/8 (25)	0.471
N. of positive mice with BM infiltration, n. (%)	1/8 (12.5)	2/8 (25)	1.000
Survival time (days)[1] of CNS positive mice	50.1±8.3	74±14.8	0.009

Positive mice are those that developed lymphoma. p values were calculated using the log rank test for survival time and Fisher's exact test for categorical variables. CNS: central nervous system; LN: lymph node; BM: bone marrow. [1]Values represent the mean ± SD.

crossing the blood-brain barrier. Most drugs included in the standard therapy for DLBCL, CHOP-R, do not reach the CNS[11,20] and, consequently, do not prevent CNS relapse. Interestingly, E7123 parent compound, celecoxib, crossed the blood-brain barrier[46] and prolonged mice survival time in a xenograft model of PCNSL.[36] The clinical use of celecoxib, however, is associated with risk of cardiovascular events associated with COX-2 inhibition.[47] E7123, in contrast, does not inhibit COX-2. Thus, E7123 is likely to be effective for the treatment of CNS relapse in DLBCL without showing cardiovascular toxicity. Considering the unproven efficacy of current drugs in CNS relapse treatment, and the physical discomfort associated with intrathecal administration, orally available E7123 could be an alternative treatment in combination with current therapy to treat CNS involvement in DLBCL in the future.

In conclusion, E7123 is a new, well tolerated and orally bioavailable therapeutic agent that increases survival time in a novel xenograft model of DLBCL with CNS involvement. We believe E7123 merits further investigation, as findings to date suggest it may improve current management and Quality of Life of DLBCL patients with this fatal condition.

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