FULL PAPER

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# Inhibition of CXCR7 extends survival following irradiation of brain tumours in mice and rats

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**Background:** In experimental models of glioblastoma multiforme (GBM), irradiation (IR) induces local expression of the chemokine CXCL12/SDF-1, which promotes tumour recurrence. The role of CXCR7, the high-affinity receptor for CXCL12, in the tumour's response to IR has not been addressed.

**Methods:** We tested CXCR7 inhibitors for their effects on tumour growth and/or animal survival post IR in three rodent GBM models. We used immunohistochemistry to determine where CXCR7 protein is expressed in the tumours and in human GBM samples. We used neurosphere formation assays with human GBM xenografts to determine whether CXCR7 is required for cancer stem cell (CSC) activity *in vitro*.

**Results:** CXCR7 was detected on tumour cells and/or tumour-associated vasculature in the rodent models and in human GBM. In human GBM, CXCR7 expression increased with glioma grade and was spatially associated with CXCL12 and CXCL11/I-TAC. In the rodent GBM models, pharmacological inhibition of CXCR7 post IR caused tumour regression, blocked tumour recurrence, and/or substantially prolonged survival. CXCR7 expression levels on human GBM xenograft cells correlated with neurosphere-forming activity, and a CXCR7 inhibitor blocked sphere formation by sorted CSCs.

**Conclusions:** These results indicate that CXCR7 inhibitors could block GBM tumour recurrence after IR, perhaps by interfering with CSCs.

Radiotherapy has a major role in the treatment of glioblastoma multiformes (GBMs). However, despite the use of high radiation doses and the combination of anticancer agents and targeted therapies, the tumours invariably recur, leading to the demise of more than 75% of the patients by 2 years. Importantly, most of the recurrences occur within the radiation field (Hochberg and Pruitt, 1980; Liang *et al*, 1991; Sneed *et al*, 1994; McDonald *et al*, 2011). Thus, any method of improving local control of the primary tumour by radiotherapy would improve the curability of GBM patients.

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One way to limit tumour growth after irradiation (IR) is blockade of revascularization. In animal models of cancer, doses of radiation in the therapeutic range block local angiogenesis (Ahn and Brown, 2008), forcing the tumour to generate new blood vessels (i.e., vasculogenesis) for its survival. In addition, IR induces the expression of CXCL12/SDF-1, a hypoxia-inducible chemokine (Kioi *et al*, 2010; Kozin *et al*, 2010). CXCL12 promotes vasculogenesis by recruiting $CD11b^+$ monocytes, which express the CXCL12 receptor CXCR4, into the tumour (Kozin *et al*, 2010). The monocytes remodel the tumour matrix via proteases, such as MMP-9 (Ahn and Brown, 2008), presumably to allow the new vessels to spread through the matrix. Treatment with an anti-CD11b antibody (Ahn *et al*, 2010) or a CXCR4 antagonist (Kioi *et al*, 2010; Kozin *et al*, 2010) inhibits tumour revascularization and growth after IR, indicating that CXCL12:CXCR4-mediated recruitment of $CD11b^+$ monocytes could be a target for post-IR control of GBM. In addition, as the monocytes themselves do not form the blood vessels (Kioi *et al*, 2010; Kozin *et al*, 2010), vasculogenesis could be blocked by therapies targeting the cell type that forms the new vessels, be it an endothelial cell, an endothelial precursor cell (EPC) (Asahara *et al*, 1997), a pericyte, and/or another cell type.

Another strategy to attenuate GBM tumour growth after IR is to target cancer stem cells (CSCs) within the tumour. CSCs are responsible for tumour propagation (Galli *et al*, 2004; Singh *et al*, 2004) and have an increased propensity to resist radiation (Bao *et al*, 2006) and chemotherapy (Liu *et al*, 2006). Interestingly, CSCs can differentiate into tumour endothelial cells (Ricci-Vitiani *et al*, 2010; Wang *et al*, 2010b). Although specific anti-CSC therapies are under development, strategies targeting CSCs in combination with conventional therapies have already shown promise in preclinical models (Wang *et al*, 2010a). CSCs express CXCR4, and addition of CXCL12 to these cells *in vitro* results in proliferative, pro-survival and self-renewal responses (Liu *et al*, 2006; Ehtesham *et al*, 2009; Gatti *et al*, 2013); moreover, these responses are blocked by CXCR4 antagonists (Gatti *et al*, 2013).

CXCR7 is, like CXCR4, a receptor for CXCL12/SDF-1, but with a 10-fold higher affinity towards it (Burns *et al*, 2006). CXCR7 is expressed on tumour cells, endothelial cells and pericytes in a wide variety of cancers, including GBM (Madden *et al*, 2004; Miao *et al*, 2007; Liu *et al*, 2010b). Although CXCR7 does not mediate chemotaxis of cells towards CXCL12 *in vitro*, CXCR7 facilitates binding to endothelial cells (Burns *et al*, 2006; Liu *et al*, 2010a; Dai *et al*, 2011; Yan *et al*, 2012) and regulates CXCL12-mediated transcytosis through the endothelium (Mazzinghi *et al*, 2008; Zabel *et al*, 2009; Dai *et al*, 2011; Yan *et al*, 2012). The role of CXCR7 in the glioma's response to IR has, until now, not been addressed.

In the present study, we tested the hypothesis that blocking the interaction of CXCL12 with CXCR7 could improve tumour control by IR. First, we used the CXCR7 inhibitor CCX771 (Zabel *et al*, 2009; Cruz-Orengo *et al*, 2011) in the U251 mouse GBM model to see if post-IR tumour recurrence could be blocked in the absence of CXCR4 antagonism. Second, we tested a novel CXCR7 inhibitor, CCX662, in what we believe is a highly realistic model of human brain cancer (Liu *et al*, 2013), autochthonous tumours in rats caused by exposure to the carcinogen ethylnitrosourea (ENU). Third, we tested whether CCX662 could prolong post-IR survival in the highly aggressive rat C6 GBM model. In the ENU and C6 models, we used immunohistochemistry to probe the tumours for CXCR7 expression. We also probed human glioma samples for the expression of CXCR7, CXCR4, CXCL12, the other CXCR7 ligand CXCL11 and the other CXCL11 receptor CXCR3. Finally, we determined whether CXCR7 is involved in the formation of neurospheres by human GBM xenograft CSCs *in vitro*. The predominance of the data suggests that CXCR7 inhibitors could be used in the post-IR treatment of GBM, by blocking the ability of CSCs to regrow the tumour.

## MATERIALS AND METHODS

**Animals and reagents.** All animal procedures were approved by Stanford's Administrative Panel on Laboratory Animal Care or the ChemoCentryx Institutional Animal Care and Use Committee, and met the standards required by the United Kingdom Coordinating Committee on Cancer Research guidelines (Workman *et al*, 2010). Nude (*nu/nu*) mice and Sprague-Dawley rats (both from Charles River, Wilmington, MA, USA) were maintained in a germ-free environment and had access to food and water *ad libitum*. Animals were removed from tumour studies when body weights decreased 20% from baseline. CXCR7-specific inhibitors CCX771 and CCX662 were generated at ChemoCentryx, Inc. Multi-grade astrocytoma and glioblastoma AccuMax arrays were purchased from Accurate Chemicals (Westbury, NY, USA) or generated from biopsies at Stanford University Medical Center (Palo Alto, CA, USA) under an IRB-approved protocol. Anti-CXCR7 mAb 11G8 (catalogue no. MAB42273), anti-CXCL12 mAb (catalogue no. MAB350) and IgG1 isotype control mAb were purchased from R&D Systems (Minneapolis, MN, USA). Anti-CXCL11 Ab (catalogue no. ab9955) and anti-CXCR3 mAb (catalogue no. ab64714) were purchased from Abcam (Cambridge, MA, USA). Anti-CXCR4 Ab (catalogue no. C3116) was purchased from Sigma (St Louis, MO, USA). Rabbit IgG and biotinylated goat anti-rabbit IgG were purchased from Jackson Immunoresearch (West Grove, PA, USA).

**Mouse U251 model.** One million U251/pFB-Luc cells were injected intracranially into 24 6-week-old nude (*nu/nu*) mice and, after allowing 3 weeks for tumour establishment, 12 of the mice were given whole-brain IR (12 Gy), as previously described (Kawakami *et al*, 2005). Immediately thereafter, six mice from each group were injected subcutaneously with $30\,mg\,kg^{-1}$ CCX771, whereas the remaining six mice from each group received the vehicle, 10% Captisol (Captisol, La Jolla, CA, USA), once daily for 3 weeks. For periodic tumour imaging, the mice were anaesthetized and injected intraperitoneally with $150\,mg\,kg^{-1}$ of D-luciferin (PerkenElmer, Waltham, MA, USA); 5–30 min later, the mice were analysed with the IVIS system coupled to Living Image acquisition and analysis software (PerkenElmer). Photon flux was calculated for each mouse by using a rectangular region of interest encompassing the head of the mouse in a prone position. This value was scaled to a comparable background value (from a luciferin-injected mouse with no tumour cells), and then normalised to the value obtained immediately after tumour cell injection (day 0), so that all mice had an arbitrary starting bioluminescence index of 100.

**Rat ENU model.** Brain tumours were induced by intra-peritoneal injection of Sprague-Dawley rats (Taconic Farms, Germantown, NY, USA) at gestational day 18 with ENU (Sigma $50\,mg\,kg^{-1}$). During each experiment, at least one control pregnant rat received saline vehicle under identical conditions. Pups were born and weaned at normal age, after which they were monitored at least weekly for signs of illness. At 115 days of age 12, rats were anaesthetised and placed in individual lead jigs with a cutout that allowed the brain to be irradiated tangentially with full shielding of the buccal cavity and the rest of the body. Whole-brain IR (20 Gy) was performed with a Phillips (Andover, MA, USA) X-ray unit operated at 200 kVp with a dose rate of $1.21\,Gy\,min^{-1}$ (20 mÅ with added filtration of 0.5 mm copper, distance from X-ray source to the target of 31 cm, and a half-value layer of 1.3 mm copper). To ensure the maximum uniformity of the dosage, the rats were turned 180° midway through the IR. Immediately after IR, each rat was implanted with a single Alzet 2004 infusion pump for delivery of $0.25\,\mu l\,hr^{-1}$ of CCX662 solution (six rats) or vehicle (six rats) for 4 weeks. For pump implantation, the rats were anaesthetised

and a mid-scapular incision was made to create a pocket in the subcutaneous tissue large enough to allow for some movement of the pump. The pump was then inserted into the pocket port-first to minimise the exposure of the compound to the incision. The mean plasma levels of CCX662 (386 + / − 89 nM), sampled 9 and 16 days post pump implantation, were greater than the $IC_{90}$ value of CCX662 for rat CXCR7 in 100% rat serum. Four rats receiving no IR or compound were included as controls.

**Rat C6 model.** One million C6 cells were injected intracranially into 24 6-week-old Sprague-Dawley rats, as previously described (Kubiatowski *et al*, 2001). After allowing 7 days for tumour establishment, 16 rats were irradiated (18 Gy) and given a subcutaneous Alzet pump exactly like the ENU-treated rats described above. The pumps delivered CCX662 (eight rats) or vehicle (eight rats) for 7 days, after which the pumps in the surviving rats were exchanged with freshly-loaded 7-day pumps. The rats surviving after these two pumps expired were maintained without compound. Eight rats receiving no IR or compound were included as controls. A second study was performed exactly as the first, containing seven IR + CCX662-treated rats, seven IR + vehicle-treated rats and 10 non-IR non-treated rats.

**Immunohistochemistry.** Tumour arrays were deparaffinized and hydrated by conventional methods, then subjected to antigen retrieval by immersion in 10 mM sodium citrate, 0.05% Tween-20 at 98 °C for 20 min, followed by slow cooling at room temperature for 20 min. The tumour arrays were then treated with an endogenous biotin blocking kit (Vector Labs, Burlingame, CA, USA) and stained with anti-CXCR7, CXCR4, CXCR3, CXCL12 or CXCL11 Abs, or mouse IgG1 or rabbit IgG control Abs, using a catalysed signal amplification IHC kit from Dako (Carpinteria, CA, USA) according to the manufacturers' directions. Antibodies were diluted to final concentrations of 10 $\mu g\,ml^{-1}$ (CXCL12), 2 $\mu g\,ml^{-1}$ (CXCR7), 1 $\mu g\,ml^{-1}$ (CXCL11, CXCR4) and 0.5 $\mu g\,ml^{-1}$ (CXCR3). The tumour arrays were counterstained with Mayer's hematoxylin (Sigma) for 5 min, rinsed with water for 3 min, and coverslips were mounted using Faramount (Dako). Staining intensity on tumour cells and tumour-associated vasculature was assessed with a semi-quantitative scoring system.

**CSC analysis.** T4121, T387, and T3832 GBM specimens were grown as previously described (Bao *et al*, 2006; Heddleston *et al*, 2009; Li *et al*, 2009; Eyler *et al*, 2011). Cells were maintained in mouse flank xenografts and processed using a papain dissociation kit (Worthington, Lakewood NJ, USA). Unenriched tumour cells were grown overnight in neurobasal medium (Life Technologies, Grand Island, NY, USA) containing B-27 supplement, 20 $ng\,ml^{-1}$ EGF, and 20 $ng\,ml^{-1}$ FGF (Life Technologies). CSCs were purified from bulk tumour cells using CD133/2 magnetic beads (Miltenyi, Auburn, CA, USA) and subsequently grown as spheres in complete neurobasal medium. For the neurosphere formation assay, spheres were dissociated with trypsin and the FACSAria II was used to place 1, 5, 10 or 20 live cells per well into 96-well plates containing complete neurobasal medium. A subset of wells contained the CXCR4 antagonist AMD3100 (1 or 10 $\mu g\,ml^{-1}$), the CXCR7 inhibitor CCX771 (100 nM or 1 $\mu$M) or its inactive chemical analogue CCX704 (100 nM or 1 $\mu$M). After 10 days of culture, spheres larger than 10 cells in diameter were counted. For the analysis of $CXCR7^{bright}$ *vs* $CXCR7^{dim}$ cells, bulk tumour cells were stained with CXCR7 mAb 11G8, then the brightest and dimmest 15% of cells were sorted and analysed for sphere formation, as described above. Data were analysed with the Walter and Eliza Hall Institute ELDA analyser (http://bioinf.wehi.edu.au/software/elda/).

**Statistical analysis.** Statistical analyses were performed by the two-tailed Student's *t*-test or one-way ANOVA to determine statistical significance. *P*-values of $<0.05$ were considered statistically significant. Kaplan–Meier curves and the log-rank test were used to compare survival times among the groups. All calculations were performed using Prism5 (GraphPad Software, La Jolla, CA, USA).

## RESULTS

**Inhibition of CXCR7 with CCX771 post IR prevents recurrence of intracranial U251 GBM tumours in nude mice.** Immunodeficient mice were injected intracranially with luciferase-expressing U251 human GBM cells. After tumour establishment, the mice were given whole-brain IR and then injected subcutaneously with CCX771 or its vehicle daily for 3 weeks. Whereas IR plus vehicle significantly slowed tumour growth, IR plus CCX771 resulted in rapid tumour regression (Figure 1). After the 3-week CCX771 treatment period ended, the tumours did not recur. CCX771 did not exhibit an anti-tumour effect in the absence of IR, suggesting that CXCR7 is involved in the tumour's response to IR.

**Inhibition of CXCR7 with CCX662 post IR prolongs the survival of rats with ENU-induced brain tumours.** Pregnant rats were injected with the carcinogen ENU on day 17 of gestation. When the progeny were 115 days old, before their brain tumours were large enough to produce neurological symptoms, the rats were given whole-brain IR. The rats were immediately implanted with subcutaneous infusion pumps containing CCX662, a novel inhibitor of CXCR7 possessing high potency and selectivity for CXCR7 (Supplementary Figure 1), or its vehicle. The pumps provided 4 weeks' worth of CCX662 at a concentration continually exceeding the compound's serum $IC_{90}$ value, that is, >90% CXCR7 inhibition. Whereas the group of non-irradiated rats had a median lifespan of 190 days and the group of vehicle-treated irradiated rats had a median lifespan of 205 days, the group of CCX662-treated irradiated rats had a median lifespan of 370 days (Figure 2). All control rats receiving IR but not ENU survived the 418 day study, indicating that the IR itself was not responsible for the rats' mortality (data not shown). Similar effects were observed in the ENU tumour model with a different CXCR7 inhibitor and route of administration (Supplementary Figure 2).

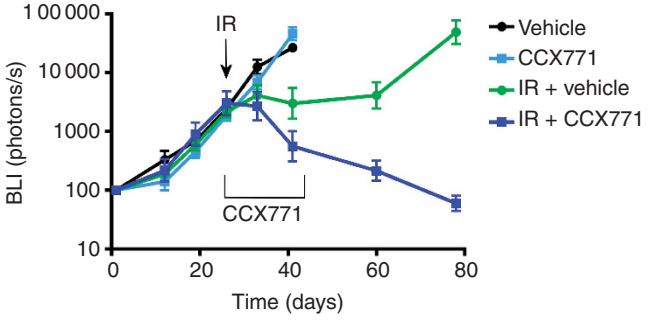


Figure 1. CCX771, in combination with IR, reduces tumour size and prevents tumour recurrence in mice containing U251 GBM tumours. U251 GBM cells were implanted intracranially into mice (day 0), and a subset of the mice was given 12 Gy whole-brain IR 3 weeks later. All mice were then treated once daily with the CXCR7 inhibitor CCX771 (30 $mg\,kg^{-1}$) or its vehicle for 3 weeks. Neither CCX771 nor the vehicle affected the tumours in non-irradiated mice. In combination with IR, however, CCX771 significantly inhibited tumour growth ($P<0.05$ compared with vehicle). The tumours in irradiated mice treated with the vehicle remained the same size for 7 weeks, but started to grow thereafter. The tumours in irradiated mice treated with CCX771 regressed over the course of the study ($n=6$ mice).

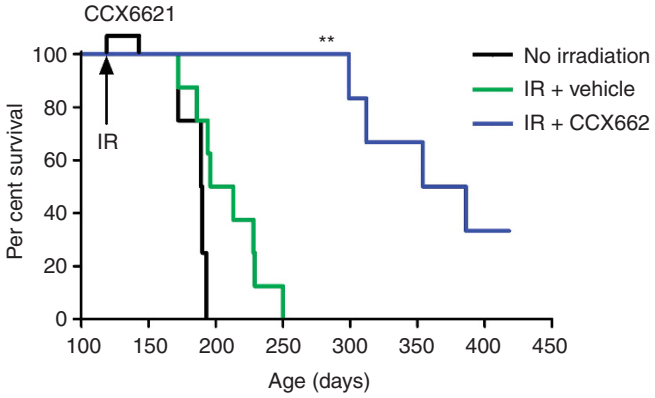


Figure 2. CCX662 increases post-IR survival time in ENU-treated rats. Rats were exposed to the carcinogen ENU *in utero* and, on day 115, given 20 Gy whole-brain IR. Immediately following IR, rats were infused with CCX662 or its vehicle for 4 weeks. CCX662 significantly increased post-IR median survival time (**$P = 0.0003$ compared with vehicle, log-rank test; $n = 4$–6 rats).

**Expression of CXCR7 in rat ENU and human GBM tumours.** To determine whether tumour cells and/or tumour-associated vasculature in ENU-induced brain tumours expressed CXCR7, we performed immunohistochemistry on the tumours on killing with the anti-CXCR7 mAb clone 11G8. CXCR7 was detected in tumour cells in most tumours from both irradiated and non-irradiated rats (Figure 3). In addition, CXCR7 was detected in a subset of blood vessels, both in the tumour and in the non-tumoural tissue. In non-tumoural tissue, CXCR7 was also detected in rare neural cells (Figure 3). To examine CXCR7 expression in human gliomas, we performed immunohistochemistry with 11G8 on a multi-grade glioma array ($n = 30$ samples). CXCR7 expression was observed in 62% (five of eight) of pilocytic astrocytomas, 88% (eight of nine) of anaplastic astrocytomas and 100% (13 or 13) of GBM tumours (Figure 4). As in the ENU-induced rat brain tumours, CXCR7 expression was noted both on tumour cells and tumour-associated vasculature. To assess the expression of CXCR7 relative to its ligands CXCL11/I-TAC and CXCL12/SDF-1, we performed immunohistochemistry on GBM-only arrays ($n = 52$ samples). We also included antibodies specific for CXCR4, the other CXCL12 receptor, and CXCR3, the other CXCL11 receptor. Whereas CXCR4 and CXCR3 were primarily expressed by tumour cells, CXCR7, CXCL12 and CXCL11 were expressed by tumour cells and tumour-associated vasculature (Figure 5; Table 1).

**Inhibition of CXCR7 with CCX662 post IR prolongs the survival of rats with C6 GBM tumours.** We sought to determine whether CXCR7 inhibition could delay post-IR growth of extremely aggressive GBM tumours. Towards this end, we used rat C6 GBM cells, which grow extremely quickly and cause death ~2 weeks post implantation. Rats were injected intracranially with C6 cells and, 7 days later, underwent whole-brain IR. Immediately after IR, the rats were infused with CCX662 or vehicle. Non-irradiated rats began succumbing to the tumours on day 10, and all rats in the non-irradiated group were dead by day 16 (Figure 6). Irradiated rats infused with vehicle began dying on day 11, and were all dead by day 18, indicating that IR does not prolong survival in this aggressive model. Remarkably, irradiated rats infused with CCX662 lived significantly longer, with median post-IR survival time increased from 6 (vehicle) to 9 days (CCX662) post IR.

**Expression of CXCR7 in rat C6 GBM tumours.** To assess CXCR7 protein expression in C6 GBM tumours, we performed immunohistochemistry on the tumours on killing with CXCR7 mAb 11G8. Although C6 GBM tumours are poorly vascularised, owing to the rapid growth of the tumour, CXCR7 was detected in a subset of blood vessels in the tumours (Figure 7). However, CXCR7 was not expressed by the tumour cells, reflecting the fact that the cell line is CXCR7$^-$ (Supplementary Figure 3).

**Expression of functional CXCR7 on human CSCs.** To investigate whether CXCR7 might be required by CSCs to regrow tumours, we performed *in vitro* neurosphere formation assays using xenografts from several human GBM specimens. First, we analysed neurosphere formation by CXCR7$^{bright}$ and CXCR7$^{dim}$ cells isolated from the xenografts. CXCR7$^{bright}$ cells exhibited a ⩾10-fold higher ability to form spheres than CXCR7$^{dim}$ cells (Figure 8A). Second, we analysed the effect of CXCR7 inhibitor CCX771 on the ability of sorted xenograft CSCs to form neurospheres in culture. CCX771, but not its inactive analogue CCX704, inhibited sphere formation in a dose-dependent manner (Figure 8B). CCX771 was more potent than AMD3100, the CXCR4 antagonist, as 1 $\mu$M CCX771 reduced neurosphere formation to a greater extent than 1 $\mu g\,ml^{-1}$ (1.3 $\mu$M) AMD3100 (Figure 8B).

## DISCUSSION

In these studies we show that CXCR7 inhibitors are effective at preventing tumour recurrence and prolonging survival after IR in rodent models of GBM. In a mouse model with intracranial tumours derived from human U251 GBM cells, IR was sufficient to block tumour growth for a number of weeks, but the tumours recurred. Daily treatment of the mice post IR with the CXCR7 inhibitor CCX771 resulted in a rapid reduction in tumour size. Tumour regression steadily continued after the 3-week CCX771 treatment period ended, with no recurrence in the final 5 weeks of follow-up. In ENU-treated rats, which develop spontaneous astrocytomas resembling human GBM tumours (Liu *et al*, 2013), IR prolonged survival only for ~2 weeks; however, 4-week treatment of the irradiated rats with the novel CXCR7 inhibitor CCX662 prolonged survival an additional ~24 weeks. Finally, in the highly aggressive rat C6 GBM model, in which IR has no effect on survival, CCX662 extended the median post-IR survival time from 6 days (vehicle) to 9 days (CXCR7 inhibitor).

To explore the mechanism through which the CXCR7 inhibitors exhibited their anti-tumour activities, we first used immunohistochemistry to determine where CXCR7 protein was expressed in the rat ENU and C6 GBM tumours, as well as human gliomas. In ENU-induced tumours, CXCR7 was detected on the majority of tumour cells and on a subset of tumour-associated blood vessels. This pattern was also seen in human gliomas, in which the incidence of CXCR7 expression on both tumour cells and tumour-associated vasculature increased with tumour grade, in agreement with prior studies (Madden *et al*, 2004; Hattermann *et al*, 2010; Liu *et al*, 2010b). In human GBM tumour arrays, CXCR7 protein was detected on tumour cells in ~50% of the samples and on the vasculature in ~70% of the samples. CXCL12 was also detected on tumour cells and tumour-associated vasculature, that is, in the same areas as CXCR7. The similarity in CXCR7 expression between ENU-induced brain tumours and human GBM tumours is not surprising, given the similarities they share in multiple other parameters (Jang *et al*, 2006; Recht *et al*, unpublished). Hence, in the ENU model, the CXCR7 inhibitor could have exerted its anti-tumour activity by acting on the tumour cells or the tumour-associated vasculature. In contrast, in C6 GBM tumours, CXCR7 protein was detected on a subset of tumour-associated blood vessels, but not on the tumour cells, reflecting the fact that the C6 cell line does not express CXCR7. This result suggests that, in the C6 model, the

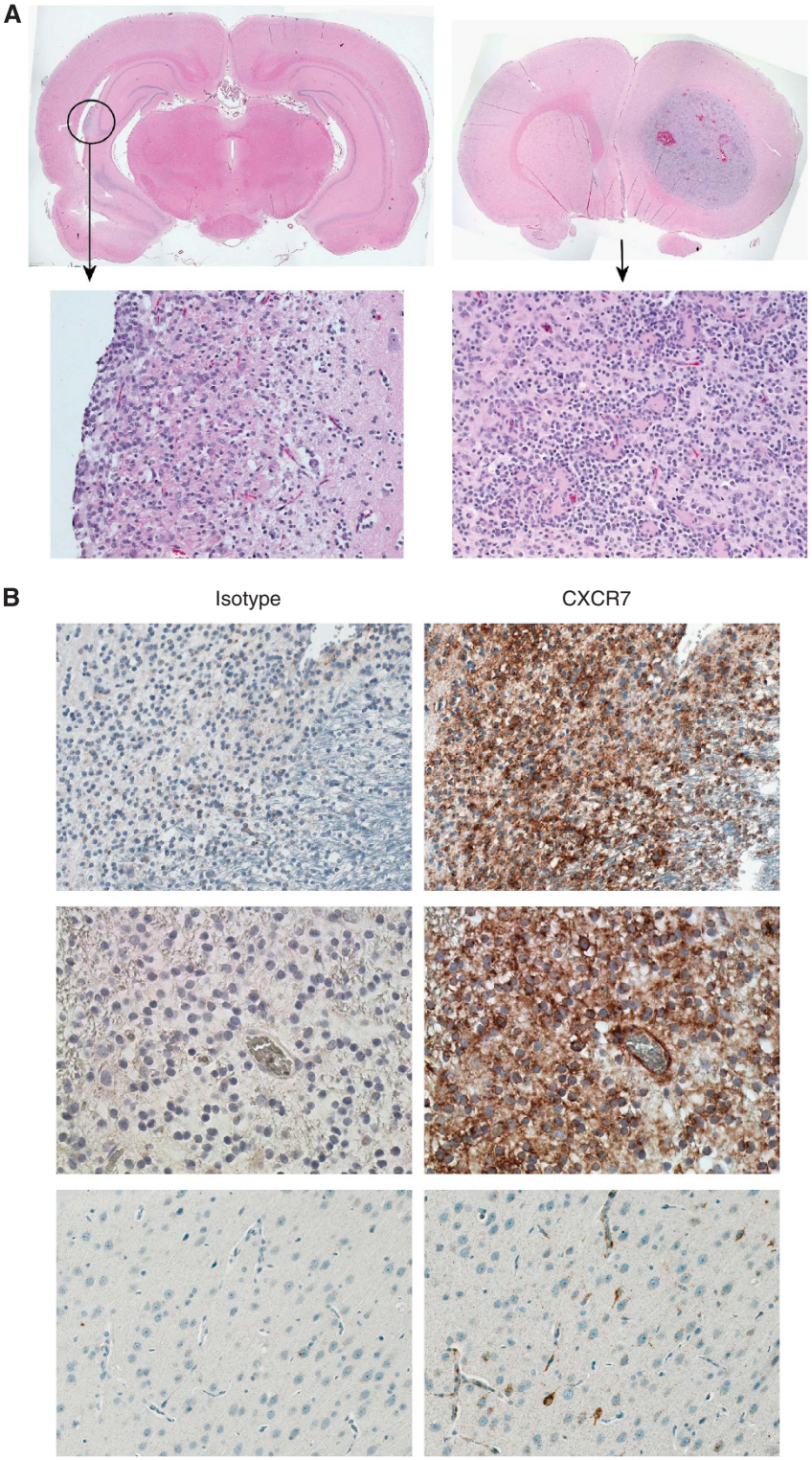


**Figure 3. CXCR7 is expressed in ENU-treated rat brain tumours.** (**A**) H&E staining of two tumours at ×20 (top row) and ×200 (bottom row) magnification. (**B**) IHC staining of two representative tumours (top and middle rows) and non-tumoural tissue (bottom row) with the CXCR7 mAb 11G8 (right column) or an isotype control mAb (left column). In tumours, 11G8 stains tumour cells and occasional blood vessels. In non-tumoural tissue, 11G8 stains occasional blood vessels and neural cells. Magnification ×200 (top and bottom rows), ×400 (middle row).

CXCR7 inhibitor exerted its anti-tumour activity by acting on the tumour vasculature or cells that form the vasculature.

To further explore the mechanism of action of the CXCR7 inhibitors, we tested them in neurosphere formation assays *in vitro*. Using xenografts from multiple human GBM specimens, we observed that a CXCR7 inhibitor reduced sphere formation from sorted $CD133^+$ CSCs in a dose-dependent manner. In separate experiments, we sorted $CXCR7^{bright}$ and $CXCR7^{dim}$ cells from total xenograft cells and found that the $CXCR7^{bright}$ cells were $\geqslant$10-fold more able than $CXCR7^{dim}$ cells to form neurospheres, indicating a correlation between CXCR7 expression level and stem activity. Although we were not able to assess CXCR7 function on CSCs in

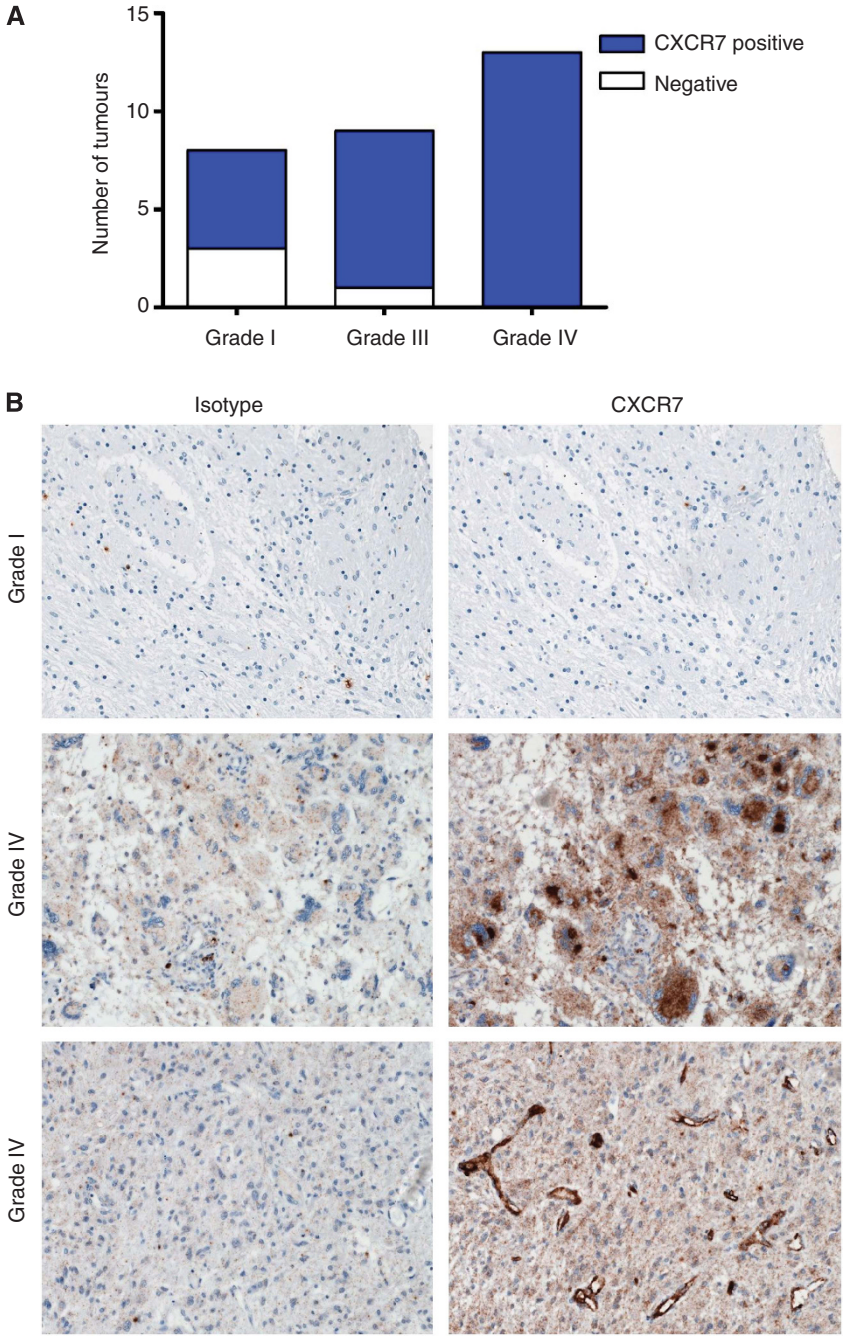


Figure 4. CXCR7 expression increases with glioma tumour grade. A multi-grade glioma array was stained by IHC with the CXCR7 mAb 11G8. (**A**) The incidence of 11G8 staining, on the tumour cells and/or tumour-associated vasculature, increased with tumour grade. (**B**) IHC staining of representative tumours with 11G8 (right column) or an isotype control mAb (left column). Note the staining of tumour cells (middle row) and tumour-associated vasculature (bottom row) in the Grade IV tumours by 11G8. Magnification $\times 200$.

the ENU and C6 models, the human GBM xenograft results raise the possibility that the CSCs in these rat models were CXCR7$^+$ and impaired by the CXCR7 inhibitors.

Together, these results indicate that CXCR7 is required for GBM tumour regrowth after IR, perhaps by facilitating the repopulation of the tumour from CSCs. Interestingly, CSCs have been shown to differentiate into endothelial cells *in vivo* (Ricci-Vitiani *et al*, 2010; Wang *et al*, 2010b), raising the possibility that CXCR7 is involved in tumour vasculogenesis after IR. Vasculogenesis could also occur via CXCR7 independently of CSCs, as CXCR7 is expressed by EPCs and mediates their CXCL12-induced adhesion to, and transcytosis through, endothelium (Dai *et al*, 2011); perhaps EPCs in blood vessels in or near the radiation field enter the CXCL12-containing tumour parenchyma via CXCR7. CXCR7 has also been shown to promote endothelial cell tube formation *in vitro* (Dai *et al*, 2011; Yan *et al*, 2012; Jin *et al*, 2013). Therefore, CXCR7 might facilitate vasculogenesis by mediating EPC trans-endothelial migration, CSC proliferation/differentiation, and/or vessel formation. CXCR7 probably does not directly participate in the IR-mediated recruitment of pro-vasculogenic CD11b$^+$ monocytes to the tumour, as these cells are CXCR7$^-$ (Berahovich *et al*, 2010) and CXCR7 inhibitors do not block trans-endothelial migration of CXCR7$^-$ cells *in vitro* (Zabel *et al*, 2009). However, CXCR7 inhibition raises the concentration of CXCL12 in the mouse bloodstream and blocks CD11b$^+$ cell migration into CXCL12-filled air pouches, presumably owing to a lessened

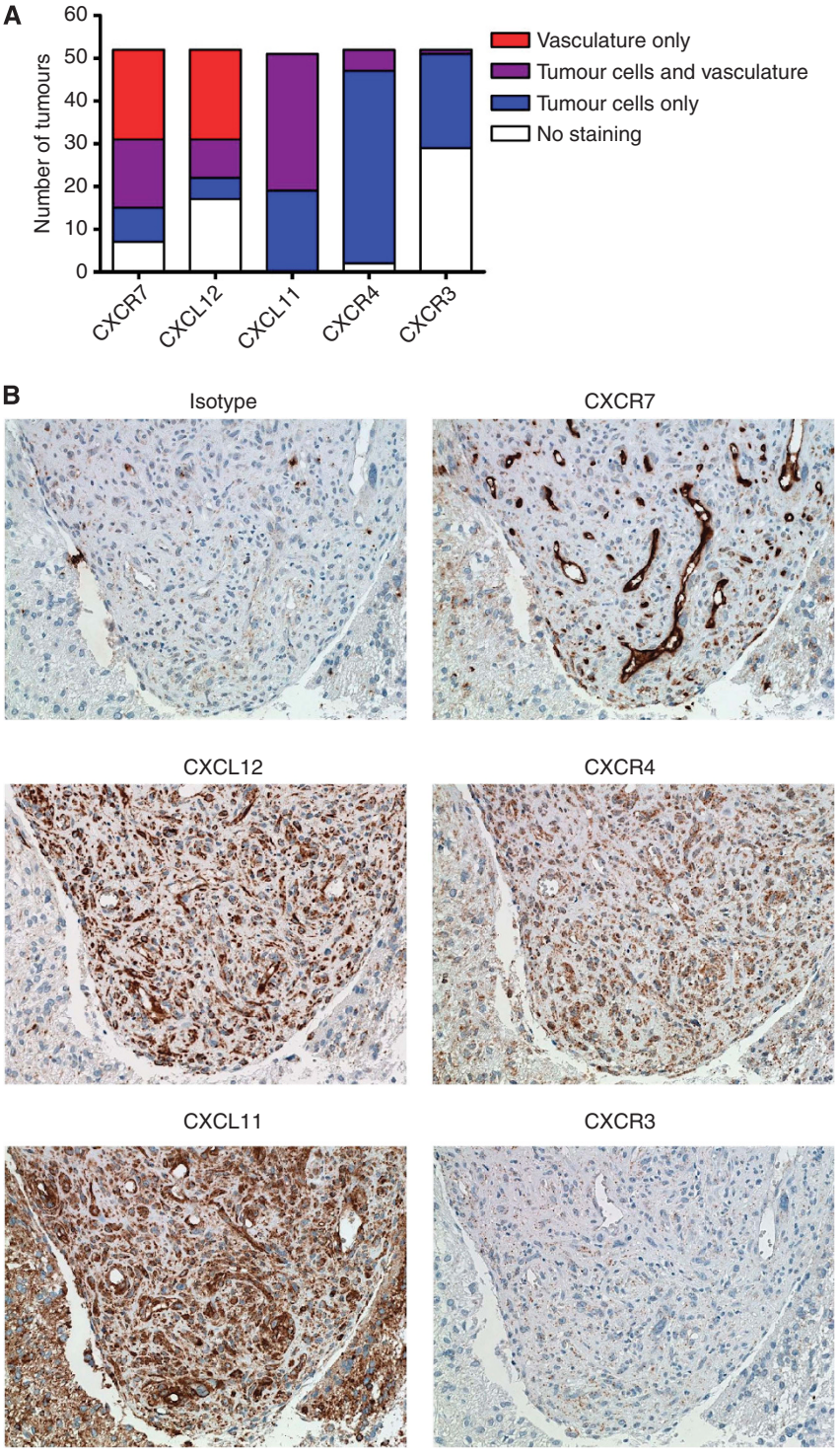


**Figure 5. CXCR7 is expressed in the vicinity of its ligands in human GBM.** GBM arrays were stained by IHC with antibodies specific for CXCR7, CXCR4, CXCR3, CXCL12 and CXCL11. (**A**) CXCR7, CXCL12 and CXCL11 were expressed on both tumour cells and tumour-associated vasculature, whereas CXCR4 and CXCR3 were expressed primarily only on tumour cells. (**B**) IHC staining of a representative GBM tumour with each antibody, as well as an isotype control antibody. In this tumour, CXCR7 is expressed primarily on the vasculature, CXCL12 and CXCL11 are expressed on the vasculature and tumour cells, CXCR4 is expressed primarily on tumour cells and CXCR3 is not expressed. Magnification $\times 200$.

CXCL12 gradient (Berahovich *et al*, 2013), raising the possibility that CXCR7 inhibitors could block $CD11b^+$ monocyte migration into irradiated tumours in an indirect manner. Indeed, CXCR7 inhibitor-mediated elevations in plasma CXCL12 levels were noted in the ENU and C6 models (Walters and Ebsworth, unpublished).

Another way CXCR7 might help irradiated tumours is through its poorly defined ability to help cells grow in stressful conditions. For example, glial cells (Zhou *et al*, 2008), endothelial cells (Gambaryan *et al*, 2011; Costello *et al*, 2012), mesenchymal stem cells (Liu *et al*, 2010a) and tumour cells (Berahovich, unpublished) upregulate CXCR7 in response to hypoxia; glioma cells are protected from temozolomide-induced apoptosis via CXCR7 (Hattermann *et al*, 2010); CXCR7 promotes endothelial cell survival (Yan *et al*, 2012; Jin *et al*, 2013); and CXCR7-transfected

Table 1. Percentage of tumours expressing chemokines or receptors on the tumour cells or tumour-associated vasculature

| | Tumour cells | Vasculature |
|---|---|---|
| CXCR7 | 46 | 71 |
| CXCL12 | 27 | 57 |
| CXCL11 | 100 | 63 |
| CXCR4 | 97 | 10 |
| CXCR3 | 44 | 2 |

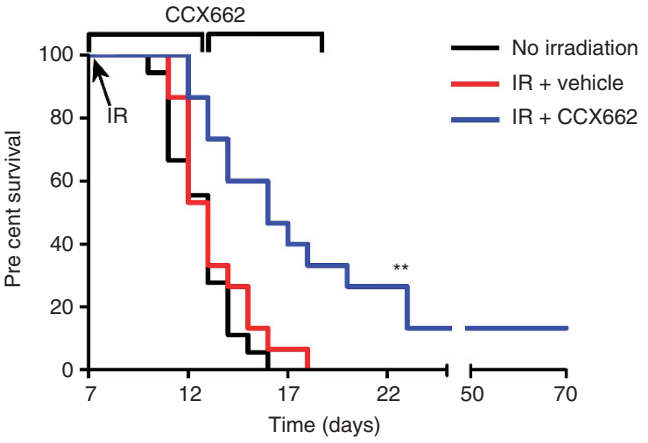


Figure 6. CCX662 increases post-IR survival time in rats containing C6 GBM tumours. C6 GBM cells were implanted intracranially into rats and, 7 days later, given 18 Gy whole-brain IR. Immediately following IR, rats were infused with CCX662 or its vehicle for 1 week. Surviving rats received another 1-week infusion of CCX662 or vehicle. CCX662 significantly increased post-IR median survival time (**$P = 0.0025$ compared with vehicle, log-rank test; $n = 15$–18 rats, compiled from two separate studies).

but not parental tumour cells can be cultured in 1% serum (Burns *et al*, 2006). In an irradiated GBM tumour, CSCs, their progeny, or endothelial cells might use CXCR7 to survive in the presence of dangerous cellular debris.

As CXCR7 can bind to both CXCL12 and CXCL11, it is possible that CXCL11 may mediate some of the activities of CXCR7 in GBM. Although we were not able to ascertain whether CXCL11 is expressed in the rodent models, owing to the lack of a suitable CXCL11 antibody, we detected CXCL11 in all 52 human GBM samples we analysed. Like CXCR7 and CXCL12, CXCL11 was expressed by both tumour cells and tumour-associated vasculature. Although CXCL11 protein expression in human GBM has heretofore not been documented, CXCL11 mRNA levels were found to be higher in GBM samples than in normal brain or low-grade glioma samples (Calatozzolo *et al*, 2011). CXCL11 protein was detected on tumour cells and tumour-associated vasculature in meningioma (Wurth *et al*, 2011), and CXCL11 mRNA or protein has been detected in a number of other solid tumours (Furuya *et al*, 2007; Lo *et al*, 2010; Monnier *et al*, 2011; Xia *et al*, 2011). If CXCL11–CXCR7 interactions have a role in GBM, CXCR7 inhibitors will block these activities, as the inhibitors potently block CXCL11 binding to CXCR7.

In summary, we have used three orthotopic rodent models of GBM to show that CXCR7 inhibitors, in combination with IR, exhibit beneficial effects on tumour size/recurrence and animal survival. The fact that the effects of CXCR7 inhibition continued after the cessation of treatment suggests that IR induces a transient CXCR7-mediated event necessary for tumour regrowth. CXCR7 expression on tumour cells and/or tumour-associated vasculature in human GBM and the rodent models, coupled with inhibition of human GBM xenograft neurosphere formation by a CXCR7 inhibitor, suggest that this event could be vasculogenesis and/or proliferation/differentiation of CSCs. The results shown herein provide substantial evidence that CXCR7 is a promising target for the post-IR treatment of GBM.

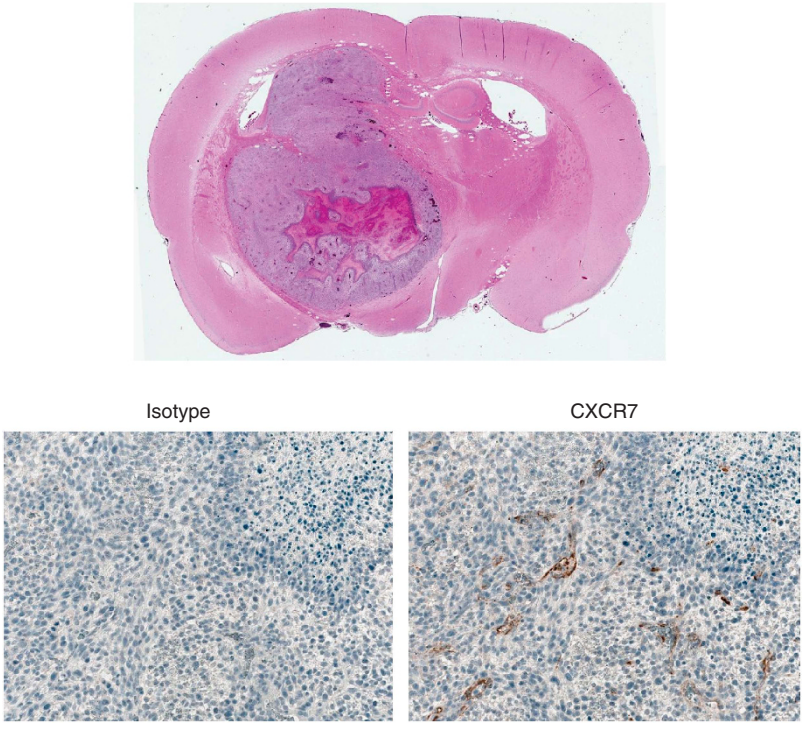


Figure 7. CXCR7 is expressed on the vasculature in C6 GBM tumours. (**A**) H&E staining of a representative tumour at ×20 magnification. (**B**) IHC staining of a representative tumour with the CXCR7 mAb 11G8 or an isotype control mAb at ×200 magnification.

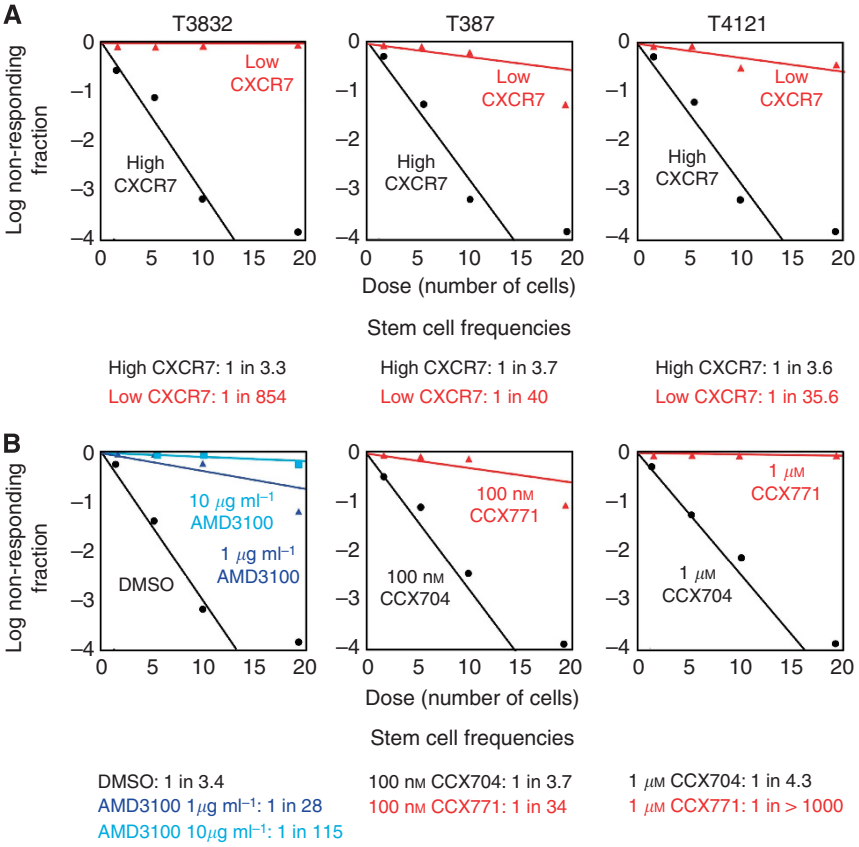


Figure 8. CXCR7 is required for CSC activity *in vitro*. (**A**) The neurosphere formation assay using CXCR7$^{bright}$ and CXCR7$^{dim}$ sorted cells from GBM xenografts T3832, T387 and T4121. CXCR7$^{bright}$ cells exhibited a greater ability to form spheres than did CXCR7$^{dim}$ cells. (**B**) Neurosphere formation assay using sorted CD133$^{+}$ CSCs from GBM xenograft T4121. CXCR7 inhibitor CCX771, but not its inactive chemical analogue CCX704, inhibited sphere formation. Inhibition of sphere formation was greater with 1 μM CCX771 than with 100 nM CX771 or with 1 μg ml$^{-1}$ (1.3 μM) AMD3100. Similar results were observed with GBM xenograft T3832 (not shown).

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## CONFLICT OF INTEREST

MJW, KE, RDB, PZ, JPP, JCJ and TJS are employees of ChemoCentryx, Inc. S-CL, RAO, MK, SBC, DT, EEM-H, MS, RMR, JDL, JK, HEKK, MM, LR and JMB declare no conflict of interest.

## REFERENCES

Ahn GO, Brown JM (2008) Matrix metalloproteinase-9 is required for tumor vasculogenesis but not for angiogenesis: role of bone marrow-derived myelomonocytic cells. *Cancer Cell* **13**: 193–205.

Ahn GO, Tseng D, Liao CH, Dorie MJ, Czechowicz A, Brown JM (2010) Inhibition of Mac-1 (CD11b/CD18) enhances tumor response to radiation by reducing myeloid cell recruitment. *Proc Natl Acad Sci USA* **107**: 8363–8368.

Asahara T, Murohara T, Sullivan A, Silver M, van der Zee R, Li T, Witzenbichler B, Schatteman G, Isner JM (1997) Isolation of putative progenitor endothelial cells for angiogenesis. *Science* **275**: 964–967.

Bao S, Wu Q, McLendon RE, Hao Y, Shi Q, Hjelmeland AB, Dewhirst MW, Bigner DD, Rich JN (2006) Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. *Nature* **444**: 756–760.

Berahovich RD, Wang Y, Jaen JC, Schall TJ (2010) CXCR7 protein is not expressed on human or mouse leukocytes. *J Immunol* **185**: 5130–5139.

Berahovich RD, Zabel BA, Lewen S, Walters MJ, Ebsworth K, Wang Y, Jaen JC, Schall TJ (2013) Endothelial expression of CXCR7 and the regulation of systemic CXCL12 levels. *Immunology* **141**(1): 111–122.

Burns JM, Summers BC, Wang Y, Melikian A, Berahovich R, Miao Z, Penfold ME, Sunshine MJ, Littman DR, Kuo CJ, Wei K, McMaster BE, Wright K, Howard MC, Schall TJ (2006) A novel chemokine receptor for SDF-1 and I-TAC involved in cell survival, cell adhesion, and tumor development. *J Exp Med* **203**: 2201–2213.

Calatozzolo C, Canazza A, Pollo B, Di Pierro E, Ciusani E, Maderna E, Salce E, Sponza V, Frigerio S, Di Meco F, Schinelli S, Salmaggi A (2011) Expression of the new CXCL12 receptor, CXCR7, in gliomas. *Cancer Biol Ther* **11**: 242–253.

Costello CM, McCullagh B, Howell K, Sands M, Belperio JA, Keane MP, Gaine S, McLoughlin P (2012) A role for the CXCL12 receptor, CXCR7, in the pathogenesis of human pulmonary vascular disease. *Eur Respir J* **39**: 1415–1424.

Cruz-Orengo L, Holman DW, Dorsey D, Zhou L, Zhang P, Wright M, McCandless EE, Patel JR, Luker GD, Littman DR, Russell JH, Klein RS (2011) CXCR7 influences leukocyte entry into the CNS parenchyma by controlling abluminal CXCL12 abundance during autoimmunity. *J Exp Med* **208**: 327–339.

Dai X, Tan Y, Cai S, Xiong X, Wang L, Ye Q, Yan X, Ma K, Cai L (2011) The role of CXCR7 on the adhesion, proliferation and angiogenesis of endothelial progenitor cells. *J Cell Mol Med* **15**: 1299–1309.

Ehtesham M, Mapara KY, Stevenson CB, Thompson RC (2009) CXCR4 mediates the proliferation of glioblastoma progenitor cells. *Cancer Lett* **274**: 305–312.

Eyler CE, Wu Q, Yan K, MacSwords JM, Chandler-Militello D, Misuraca KL, Lathia JD, Forrester MT, Lee J, Stamler JS, Goldman SA, Bredel M, McLendon RE, Sloan AE, Hjelmeland AB, Rich JN (2011) Glioma stem cell proliferation and tumor growth are promoted by nitric oxide synthase-2. *Cell* **146**: 53–66.

Furuya M, Suyama T, Usui H, Kasuya Y, Nishiyama M, Tanaka N, Ishiwata I, Nagai Y, Shozu M, Kimura S (2007) Up-regulation of CXC chemokines and their receptors: implications for proinflammatory microenvironments of ovarian carcinomas and endometriosis. *Hum Pathol* **38**: 1676–1687.

Galli R, Binda E, Orfanelli U, Cipelletti B, Gritti A, De Vitis S, Fiocco R, Foroni C, Dimeco F, Vescovi A (2004) Isolation and characterization of tumorigenic, stem-like neural precursors from human glioblastoma. *Cancer Res* **64**: 7011–7021.

Gambaryan N, Perros F, Montani D, Cohen-Kaminsky S, Mazmanian M, Renaud JF, Simonneau G, Lombet A, Humbert M (2011) Targeting of c-kit+ haematopoietic progenitor cells prevents hypoxic pulmonary hypertension. *Eur Respir J* **37**: 1392–1399.

Gatti M, Pattarozzi A, Bajetto A, Wurth R, Daga A, Fiaschi P, Zona G, Florio T, Barbieri F (2013) Inhibition of CXCL12/CXCR4 autocrine/paracrine loop reduces viability of human glioblastoma stem-like cells affecting self-renewal activity. *Toxicology* **314**: 209–220.

Hattermann K, Held-Feindt J, Lucius R, Muerkoster SS, Penfold ME, Schall TJ, Mentlein R (2010) The chemokine receptor CXCR7 is highly expressed in human glioma cells and mediates antiapoptotic effects. *Cancer Res* **70**: 3299–3308.

Heddleston JM, Li Z, McLendon RE, Hjelmeland AB, Rich JN (2009) The hypoxic microenvironment maintains glioblastoma stem cells and promotes reprogramming towards a cancer stem cell phenotype. *Cell Cycle* **8**: 3274–3284.

Hochberg FH, Pruitt A (1980) Assumptions in the radiotherapy of glioblastoma. *Neurology* **30**: 907–911.

Jang T, Savarese T, Low HP, Kim S, Vogel H, Lapointe D, Duong T, Litofsky NS, Weimann JM, Ross AH, Recht L (2006) Osteopontin expression in intratumoral astrocytes marks tumor progression in gliomas induced by prenatal exposure to N-ethyl-N-nitrosourea. *Am J Pathol* **168**: 1676–1685.

Jin J, Zhao WC, Yuan F (2013) CXCR7/CXCR4/CXCL12 axis regulates the proliferation, migration, survival and tube formation of choroid-retinal endothelial cells. *Ophthalmic Res* **50**: 6–12.

Kawakami K, Kioi M, Liu Q, Kawakami M, Puri RK (2005) Evidence that IL-13R alpha2 chain in human glioma cells is responsible for the antitumor activity mediated by receptor-directed cytotoxin therapy. *J Immunother* **28**: 193–202.

Kioi M, Vogel H, Schultz G, Hoffman RM, Harsh GR, Brown JM (2010) Inhibition of vasculogenesis, but not angiogenesis, prevents the recurrence of glioblastoma after irradiation in mice. *J Clin Invest* **120**: 694–705.

Kozin SV, Kamoun WS, Huang Y, Dawson MR, Jain RK, Duda DG (2010) Recruitment of myeloid but not endothelial precursor cells facilitates tumor regrowth after local irradiation. *Cancer Res* **70**: 5679–5685.

Kubiatowski T, Jang T, Lachyankar MB, Salmonsen R, Nabi RR, Quesenberry PJ, Litofsky NS, Ross AH, Recht LD (2001) Association of increased phosphatidylinositol 3-kinase signaling with increased invasiveness and gelatinase activity in malignant gliomas. *J Neurosurg* **95**: 480–488.

Li Z, Bao S, Wu Q, Wang H, Eyler C, Sathornsumetee S, Shi Q, Cao Y, Lathia J, McLendon RE, Hjelmeland AB, Rich JN (2009) Hypoxia-inducible factors regulate tumorigenic capacity of glioma stem cells. *Cancer Cell* **15**: 501–513.

Liang BC, Thornton AF Jr, Sandler HM, Greenberg HS (1991) Malignant astrocytomas: focal tumor recurrence after focal external beam radiation therapy. *J Neurosurg* **75**: 559–563.

Liu G, Yuan X, Zeng Z, Tunici P, Ng H, Abdulkadir IR, Lu L, Irvin D, Black KL, Yu JS (2006) Analysis of gene expression and chemoresistance of CD133+ cancer stem cells in glioblastoma. *Mol Cancer* **5**: 67.

Liu H, Xue W, Ge G, Luo X, Li Y, Xiang H, Ding X, Tian P, Tian X (2010a) Hypoxic preconditioning advances CXCR4 and CXCR7 expression by activating HIF-1alpha in MSCs. *Biochem Biophys Res Commun* **401**: 509–515.

Liu S-C, Alomran R, Chernikova SB, Lartey F, Stafford J, Jang T, Merchant M, Zboralski D, Zöllner S, Kruschinski A, Klussmann S, Recht L, Brown JM (2013) Blockade of SDF-1 after irradiation inhibits tumor recurrences of autochthonous brain tumors in rats. *Neuro Oncol* **16**(1): 21–28.

Liu Y, Carson-Walter EB, Cooper A, Winans BN, Johnson MD, Walter KA (2010b) Vascular gene expression patterns are conserved in primary and metastatic brain tumors. *J Neurooncol* **99**: 13–24.

Lo BK, Yu M, Zloty D, Cowan B, Shapiro J, McElwee KJ (2010) CXCR3/ligands are significantly involved in the tumorigenesis of basal cell carcinomas. *Am J Pathol* **176**: 2435–2446.

Madden SL, Cook BP, Nacht M, Weber WD, Callahan MR, Jiang Y, Dufault MR, Zhang X, Zhang W, Walter-Yohrling J, Rouleau C, Akmaev VR, Wang CJ, Cao St X, Martin TB, Roberts BL, Teicher BA, Klinger KW, Stan RV, Lucey B, Carson-Walter EB, Laterra J, Walter KA (2004) Vascular gene expression in nonneoplastic and malignant brain. *Am J Pathol* **165**: 601–608.

Mazzinghi B, Ronconi E, Lazzeri E, Sagrinati C, Ballerini L, Angelotti ML, Parente E, Mancina R, Netti GS, Becherucci F, Gacci M, Carini M, Gesualdo L, Rotondi M, Maggi E, Lasagni L, Serio M, Romagnani S, Romagnani P (2008) Essential but differential role for CXCR4 and CXCR7 in the therapeutic homing of human renal progenitor cells. *J Exp Med* **205**: 479–490.

McDonald MW, Shu HK, Curran Jr WJ, Crocker IR (2011) Pattern of failure after limited margin radiotherapy and temozolomide for glioblastoma. *Int J Radiat Oncol Biol Phys* **79**: 130–136.

Miao Z, Luker KE, Summers BC, Berahovich R, Bhojani MS, Rehemtulla A, Kleer CG, Essner JJ, Nasevicius A, Luker GD, Howard MC, Schall TJ (2007) CXCR7 (RDC1) promotes breast and lung tumor growth in vivo and is expressed on tumor-associated vasculature. *Proc Natl Acad Sci USA* **104**: 15735–15740.

Monnier J, Boissan M, L'Helgoualc'h A, Lacombe ML, Turlin B, Zucman-Rossi J, Theret N, Piquet-Pellorce C, Samson M (2011) CXCR7 is up-regulated in human and murine hepatocellular carcinoma and is specifically expressed by endothelial cells. *Eur J Cancer* **48**(1): 138–148.

Ricci-Vitiani L, Pallini R, Biffoni M, Todaro M, Invernici G, Cenci T, Maira G, Parati EA, Stassi G, Larocca LM, De Maria R (2010) Tumour vascularization via endothelial differentiation of glioblastoma stem-like cells. *Nature* **468**: 824–828.

Singh SK, Hawkins C, Clarke ID, Squire JA, Bayani J, Hide T, Henkelman RM, Cusimano MD, Dirks PB (2004) Identification of human brain tumour initiating cells. *Nature* **432**: 396–401.

Sneed PK, Gutin PH, Larson DA, Malec MK, Phillips TL, Prados MD, Scharfen CO, Weaver KA, Wara WM (1994) Patterns of recurrence of glioblastoma multiforme after external irradiation followed by implant boost. *Int J Radiat Oncol Biol Phys* **29**: 719–727.

Wang J, Wakeman TP, Lathia JD, Hjelmeland AB, Wang XF, White RR, Rich JN, Sullenger BA (2010a) Notch promotes radioresistance of glioma stem cells. *Stem Cells* **28**: 17–28.

Wang R, Chadalavada K, Wilshire J, Kowalik U, Hovinga KE, Geber A, Fligelman B, Leversha M, Brennan C, Tabar V (2010b) Glioblastoma stem-like cells give rise to tumour endothelium. *Nature* **468**: 829–833.

Workman P, Aboagye EO, Balkwill F, Balmain A, Bruder G, Chaplin DJ, Double JA, Everitt J, Farningham DA, Glennie MJ, Kelland LR, Robinson V, Stratford IJ, Tozer GM, Watson S, Wedge SR, Eccles SA (2010) Guidelines for the welfare and use of animals in cancer research. *Br J Cancer* **102**: 1555–1577.

Wurth R, Barbieri F, Bajetto A, Pattarozzi A, Gatti M, Porcile C, Zona G, Ravetti JL, Spaziante R, Florio T (2011) Expression of CXCR7 chemokine receptor in human meningioma cells and in intratumoral microvasculature. *J Neuroimmunol* **234**: 115–123.

Xia J, Wang J, Chen N, Dai Y, Hong Y, Chen X, Cheng B (2011) Expressions of CXCR7/ligands may be involved in oral carcinogenesis. *J Mol Histol* **42**: 175–180.

Yan X, Cai S, Xiong X, Sun W, Dai X, Chen S, Ye Q, Song Z, Jiang Q, Xu Z (2012) Chemokine receptor CXCR7 mediates human endothelial progenitor cells survival, angiogenesis, but not proliferation. *J Cell Biochem* **113**: 1437–1446.

Zabel BA, Wang Y, Lewen S, Berahovich RD, Penfold ME, Zhang P, Powers J, Summers BC, Miao Z, Zhao B, Jalili A, Janowska-Wieczorek A, Jaen JC, Schall TJ (2009) Elucidation of CXCR7-mediated signaling events and inhibition of CXCR4-mediated tumor cell transendothelial migration by CXCR7 ligands. *J Immunol* **183**: 3204–3211.

Zhou D, Wang J, Zapala MA, Xue J, Schork NJ, Haddad GG (2008) Gene expression in mouse brain following chronic hypoxia: role of sarcospan in glial cell death. *Physiol Genomics* **32**: 370–379.



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