

Oncolytic Herpes Simplex Virus and PI3K Inhibitor BKM120 Synergize to Promote Killing of Prostate Cancer Stem-like Cells

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Novel therapies to override chemo-radiation resistance in prostate cancer (PCa) are needed. Prostate cancer sphere-forming cells (PCSCs) (also termed prostate cancer stem-like cells) likely participate in tumor progression and recurrence, and they are important therapeutic targets. We established PCSC-enriched spheres by culturing human (DU145) and murine (TRAMP-C2) PCa cells in growth factor-defined serum-free medium, and we characterized stem-like properties of clonogenicity and tumorigenicity. The efficacy of two different oncolytic herpes simplex viruses (oHSVs) (G47Δ and MG18L) in PCSCs was tested alone and in combination with radiation; chemotherapy; and inhibitors of phosphoinositide 3-kinase (PI3K), Wnt, and NOTCH *in vitro*; and, G47Δ was tested with the PI3K inhibitor BKM120 in a PCSC-derived tumor model *in vivo*. PCSCs were more tumorigenic than serum-cultured parental cells. Human and murine PCSCs were sensitive to oHSV and BKM120 killing *in vitro*, while the combination was synergistic. oHSV combined with radiation, docetaxel, Wnt, or NOTCH inhibitors was not. In athymic mice bearing DU145 PCSC-derived tumors, the combination of intra-tumoral G47Δ and systemic BKM120 induced complete regression of tumors in 2 of 7 animals, and it exhibited superior anti-tumor activity compared to either monotherapy alone, with no detectable toxicity. oHSV synergizes with BKM120 in killing PCSCs *in vitro*, and the combination markedly inhibits tumor growth, even inducing regression *in vivo*.

INTRODUCTION

It is estimated that there were almost 1.3 million new cases of prostate cancer and 359,000 associated deaths worldwide in 2018, ranking as the second most frequent cancer and the fifth leading cause of cancer death in men.¹ Radical prostatectomy, hormone therapy, chemotherapy, and radiotherapy are effective in the initial phase of the disease, but prostate cancer (PCa) can eventually progress to invasive, drug- and castration-resistant metastatic disease.² The cancer stem cell hypothesis posits that a subpopulation of cancer cells drives tumor growth and metastasis and is more resistant to chemo-radiotherapy than differentiated daughter cells.³ Prostate cancer sphere-forming (or stem-like) cells (PCSCs) have been isolated from human PCa biopsies and cell lines, and they self-renew *in vitro*.^{4,5} PCSCs are

thought to be important in PCa progression, heterogeneity, recurrence, and resistance to therapy.⁵ We define PCSCs physiologically as cells that can proliferate and self-renew *in vitro* as spheroids in the absence of serum and initiate tumors *in vivo* with increased efficiency.

Oncolytic herpes simplex viruses (oHSVs) are genetically engineered HSV that can selectively replicate in and kill cancer cells, amplifying themselves and spreading within tumors, but sparing normal tissue.⁶ oHSV has been safely used in human subjects with a variety of cancers,⁶ including PCa.⁷ Here we used 2 genetically distinct oHSVs: G47Δ, with deletions of both copies of the γ 34.5 gene, a lacZ insertion inactivating the UL39 gene, and a deletion within the α 47 gene;⁸ and MG18L, with a US3 deletion and an inactivating LacZ insertion in UL39.⁹ The safety of oHSVs for cancer therapy has been demonstrated in numerous clinical trials, and one oHSV, talimogene laherparepvec (Imlygic), is approved for the treatment of advanced melanoma.⁶ Our previous work demonstrated target specificity and replication competence of oHSV in human PCa cell lines and tumor specimens.^{10–12} We herein demonstrate the efficacy of oHSV in PCSCs and PCSC-derived tumors.

oHSV can interact synergistically with other therapeutic modalities.^{13,14} We combined oHSV with chemotherapy, radiotherapy, and cancer stem cell pathway inhibitors to identify potentially synergistic interactions. We found that oHSV synergizes with the phosphoinositide 3-kinase (PI3K) pathway inhibitor BKM120 (Buparlisib) in killing PCSCs *in vitro* and *in vivo*. BKM120 is a potent and highly specific oral pan-class I PI3K inhibitor that does not inhibit mechanistic target of rapamycin (mTOR) and Vps34 kinases.¹⁵ However, in clinical trial, BKM120 was not effective against PCa.¹⁶

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RESULTS

PCSCs Have High Self-Renewal Potentiality *In Vitro*

We cultured human DU145 prostate cancer cells in growth factor-defined (epidermal growth factor [EGF] and fibroblast growth factor [FGF]-2) serum-free (GFDSF) medium for 3 weeks. When 100 cells/well were plated, 17% of wells had spheres, while no spheres were observed at 10 cells/well (Figure 1A). DU145 cells cultured in GFDSF medium for 3 months (PCSCs) had higher self-renewal potential, so that 58% of wells plated with 1 cell/well had spheres (Figure 1B).

Extreme limiting dilution analysis (ELDA) comparing 3-week to 3-month DU145 spheres demonstrated that 3-week spheres were estimated to have a 1/325 repopulating cell frequency, while 3-month-cultured DU145 spheres were estimated to have a 1/1.7 repopulating cell frequency (Figure 1C). The 3-month-cultured spheres when plated at 1 cell/well were larger than any of the parental 3-week spheres (Figures 1D–1F), suggesting greater proliferation. When mouse parental TRAMP-C2 (serum-cultured) PCa cells were assayed for sphere formation, 3.3% of wells had spheres in the 10 cells/well group (Figure 1G). In contrast, TRAMP-C2 cells grown in GFDSF medium for 3 months (PCSCs) had spheres in 22% of wells at 1 cell/well (Figure 1H). ELDA comparing TRAMP-C2 parental cells with sphere-cultured cells indicated that TRAMP-C2 parental cells had a 1/121 repopulating cell frequency, while sphere-cultured cells had a 1/3.8 repopulating cell frequency (Figure 1I). Sphere diameters for TRAMP-C2 sphere culture cells and parental cells were similar (Figures 1J–1L).

Radiation and Docetaxel Sensitivity of PCSCs and Parental Cells *In Vitro*

Cancer stem-like cells have been shown to exhibit resistance to radiotherapy and chemotherapy.^{2,17} We examined whether PCSCs have a differential response to these modalities compared with parental cells. Spheres and parental cells were treated with various doses of radiation or docetaxel *in vitro*. Both DU145 and TRAMP-C2 PCSCs were more radiotherapy resistant than parental cells (Figures 2A and 2C). DU145 PCSCs had similar sensitivity to docetaxel as parental cells (Figure 2B), while TRAMP-C2 PCSCs were much more docetaxel resistant than parental cells (Figure 2D).

PCSCs Are More Tumorigenic Than Parental Cells

We compared the tumorigenicity of PCSCs after subcutaneous implantation. It took at least 5×10^5 implanted DU145 parental cells before tumors were observed in nude mice, with two of three tumors regressing after initiation (Figure 3A). In contrast, DU145 PCSCs formed tumors with only 5×10^2 cells in all three mice tested (Figure 3C). Greater than 1×10^4 TRAMP-C2 parental cells were required to form tumors in syngeneic C57BL/6 mice (Figure 3B), while only 1×10^2 PCSCs formed tumors in all three mice (Figure 3D). Thus, PCSCs have a 1,000-fold increased tumorigenic potential *in vivo* relative to their parental counterparts.

Histologic evaluation was performed on tumors from selected animals when the tumor diameter reached 15 mm. With H&E staining, there was no difference in the morphology between TRAMP-C2 or DU145 PCSC-derived tumors and their parental cell-derived tumors (Figures 3E and 3F). However, the percentage of Ki67-positive cells was higher in both the TRAMP-C2 and the DU145 PCSC-derived tumors compared to their parental counterparts (Figures 3E and 3F).

PCSCs Are Sensitive to oHSV *In Vitro*

We previously showed that oHSV kills PCa cell lines and selectively infected PCa cells in human organotypic cultures.^{10,18,19} Therefore, we determined whether PCSCs were sensitive to oHSV. We first used G47 Δ -mCherry to evaluate virus spread in infected DU145 spheres with and without heparin at 24, 48, and 72 h after infection. Heparin inhibited oHSV infection and spread; mCherry expression only increased in the absence of heparin (Figure S1A). The presence of heparin in PCSC media inhibited oHSV killing of DU145 PCSCs (Figures S1B and S1C). However, in heparin-free medium, DU145 PCSCs were very sensitive to killing by G47 Δ and MG18L, with half-maximum inhibitory concentration (IC₅₀) values of 0.009 and 0.021 MOI, respectively (Figures S1D, 4A, and S2A). Thereafter, all virus infections were done in heparin-free medium. TRAMP-C2 PCSCs were less but similarly sensitive to both viruses, like most mouse cancer cells, with IC₅₀ values of 0.35 and 0.27 MOI, respectively (Figures 4A and S2B).

oHSV Synergizes with PI3K Inhibitor BKM120 to Kill PCSCs *In Vitro*

Several therapeutic agents were tested in combination with oHSV. oHSV combined with chemotherapy (docetaxel or cisplatin) (Figure S3A) or radiation (Figure S3B) did not exhibit combination effects *in vitro*. DU145 and TRAMP-C2 PCSCs were not very sensitive to molecularly targeted inhibitors of the NOTCH (gamma-secretase inhibitor [GSI]) and Wnt (ICG001) pathways, which was not increased with oHSV (G47 Δ and MG18L) (Figures S3C and S3D). In contrast, both DU145 and TRAMP-C2 PCSCs and their parental cells were sensitive to PI3K pathway inhibition with BKM120 (Figure 4B). Moreover, combining BKM120 with either G47 Δ or MG18L was synergistic in DU145 and TRAMP-C2 PCSCs (Figures 4C and 4D). The addition of BKM120 at 0.2 μ M (~IC₁₀) to DU145 PCSCs did not increase the ability of G47 Δ to replicate at either of two MOIs (0.1 and 1.5) (Figure 4E). Western blot demonstrated oHSV-induced phosphorylation of AKT in both DU145 and TRAMP-C2 PCSCs, which was inhibited by BKM120 (Figure 4F). In TRAMP-C2, oHSV induced cleavage of caspase-3, indicative of apoptosis, which was not increased by BKM120 (Figure 4F).

Combination of G47 Δ with PI3K Inhibitor BKM120 *In Vivo*

We tested the efficacy of monotherapy and combination therapy in subcutaneous DU145 PCSC-derived tumors. The schema is illustrated in (Figure 5A). We used G47 Δ because it is already in clinical trial for primary prostate cancer and glioblastoma. Thus, we felt that data using G47 Δ could relatively rapidly progress to clinical trial. In contrast, MG18L is a laboratory construct with no

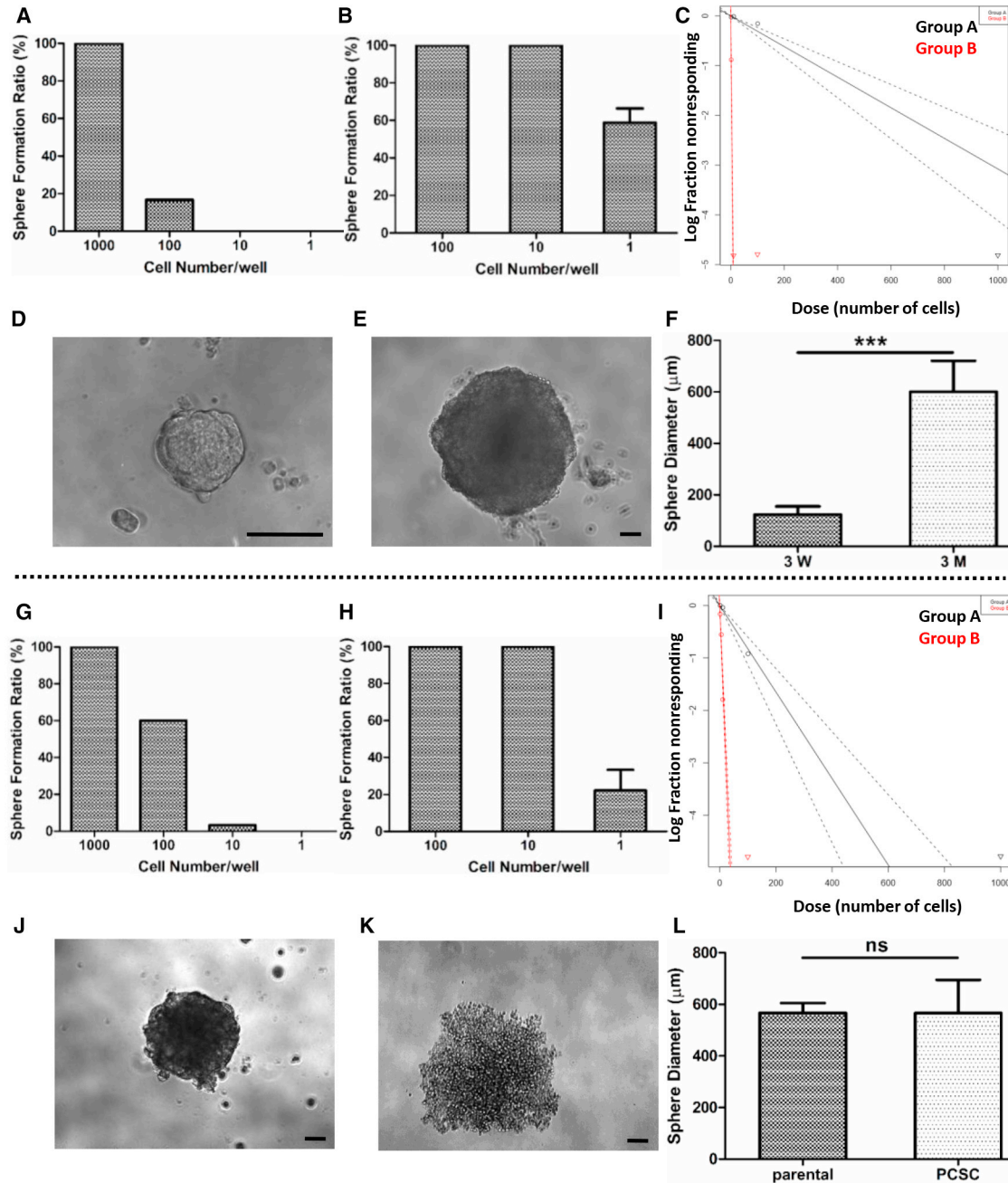


Figure 1. PCSCs Have Self-Renewal Potential *In Vitro*

(A) Sphere formation assay in 3-week DU145 sphere-cultured cells (n = 60 wells). (B) Sphere formation assay in 3-month DU145 sphere-cultured cells. For 1 cell/well assay, experiment was repeated 4 times. (C) Extreme limiting dilution analysis (ELDA) comparing 3-week DU145 sphere cultures (group A, black) and 3-month DU145 sphere cultures (group B, red) ($p < 0.001$). (D) Morphology of 3-week DU145 sphere plated at 1,000 cells/well. (E) Morphology of 3-month DU145 sphere plated at 1 cell/well. (F) Comparison of sphere diameters between 3-week DU145 spheres (1,000 cells/well) and 3-month DU145 spheres (1 cell/well). n = 3, mean ± SD, *** $p < 0.0001$. (G) Sphere formation assay of TRAMP-C2 parental cells (n = 60 wells). (H) Sphere formation assay of TRAMP-C2 sphere-cultured cells. For 1 cell/well assay, experiment was repeated 3 times. (I) ELDA comparing TRAMP-C2 parental (group A, black) and TRAMP-C2 sphere-cultured cells (group B, red) ($p < 0.001$). (J) Morphology of sphere in 100 cells/well of TRAMP-C2 parental. (K) Morphology of sphere in 1 cell/well of TRAMP-C2 sphere culture. (L) Comparison of sphere diameters between TRAMP-C2 parental (100 cells/well) and TRAMP-C2 sphere cultures (1 cell/well). There is no statistical difference. Scale bars, 100 μm.

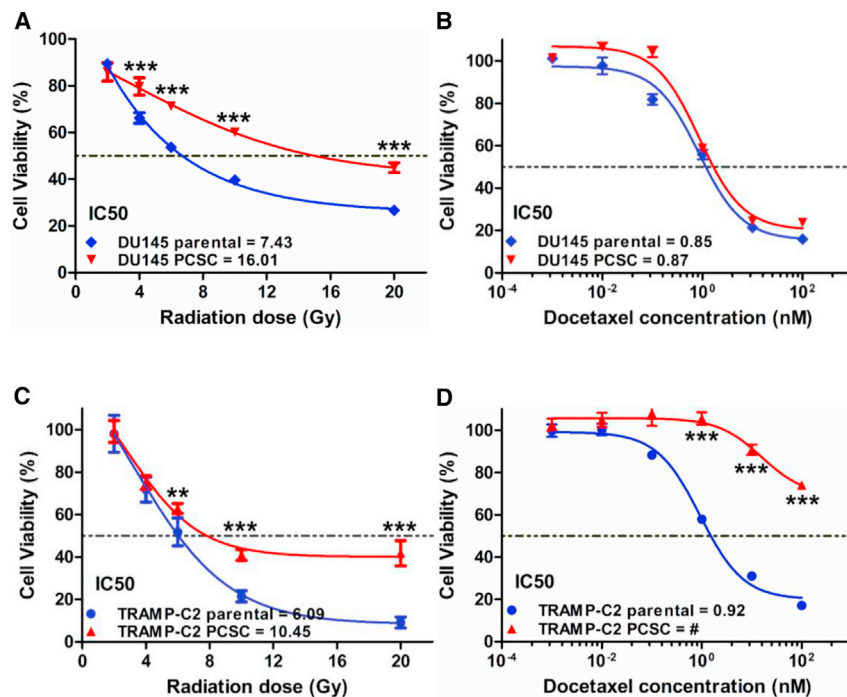


Figure 2. DU145 and TRAMP-C2 PCSCs Are Less Sensitive to Radiotherapy Than Parental Cells

Cell viability of DU145 cells after irradiation (A) and docetaxel (B) and TRAMP-C2 cells after irradiation (C) and docetaxel (D). Viability was normalized to no treatment. #no IC₅₀. Data are shown as mean \pm SD. **p < 0.01, ***p < 0.001.

clinical efficacy or safety demonstrated thus far. Combination treatment (G47 Δ and BKM120) induced complete, durable (3-month) tumor regression in 2 of 7 animals, and it reduced tumor growth in the others (Figure 5B). Both G47 Δ and BKM120 monotherapy delayed tumor growth, but the combination was significantly better than either monotherapy (Figures 5B and 5C). No toxicity due to monotherapy or combination therapy was observed. We documented normal histopathology and stable weight (Figures S4A and S4B). There was no fatty degeneration of liver, pulmonary or splenic congestion, cardiac muscle cell hypertrophy, or glomerular atrophy in either the single-treatment or combination group (Figure S4A).

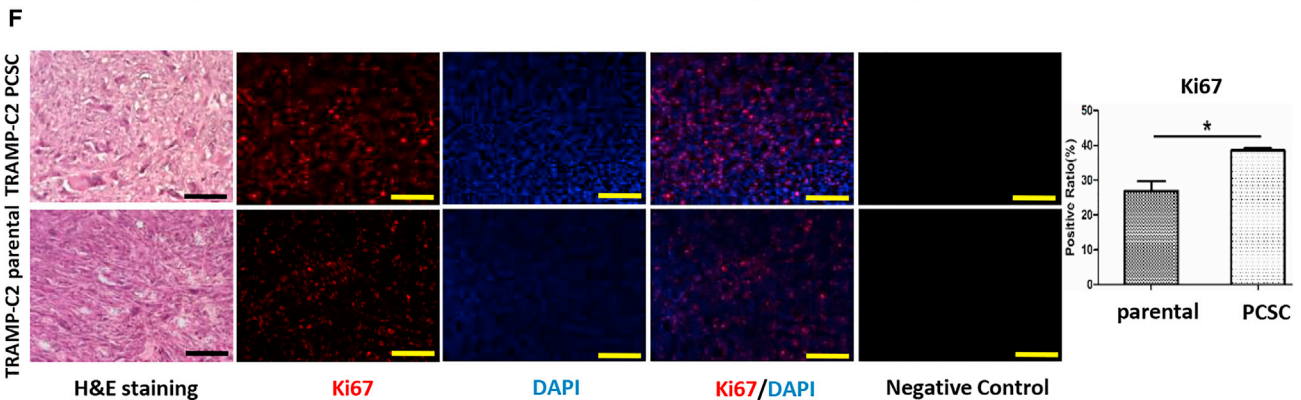
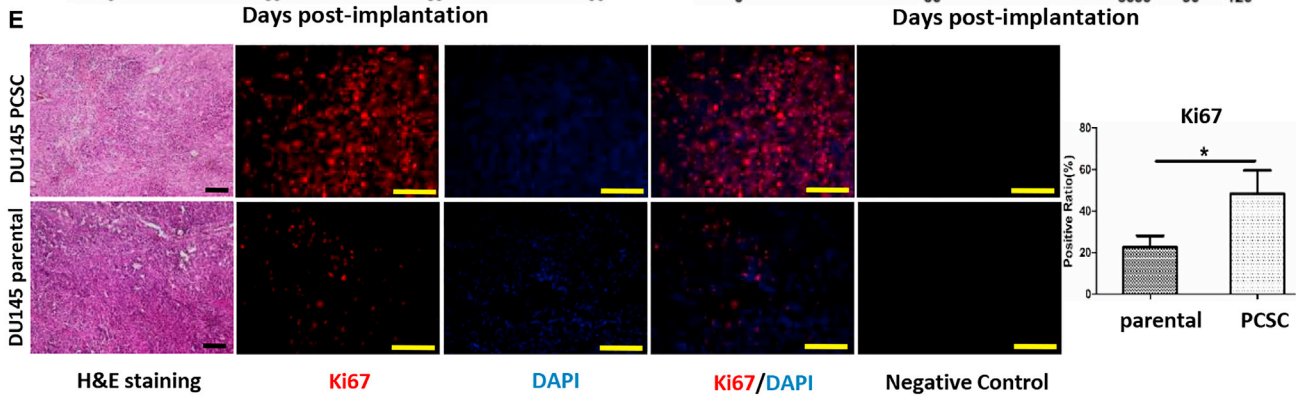
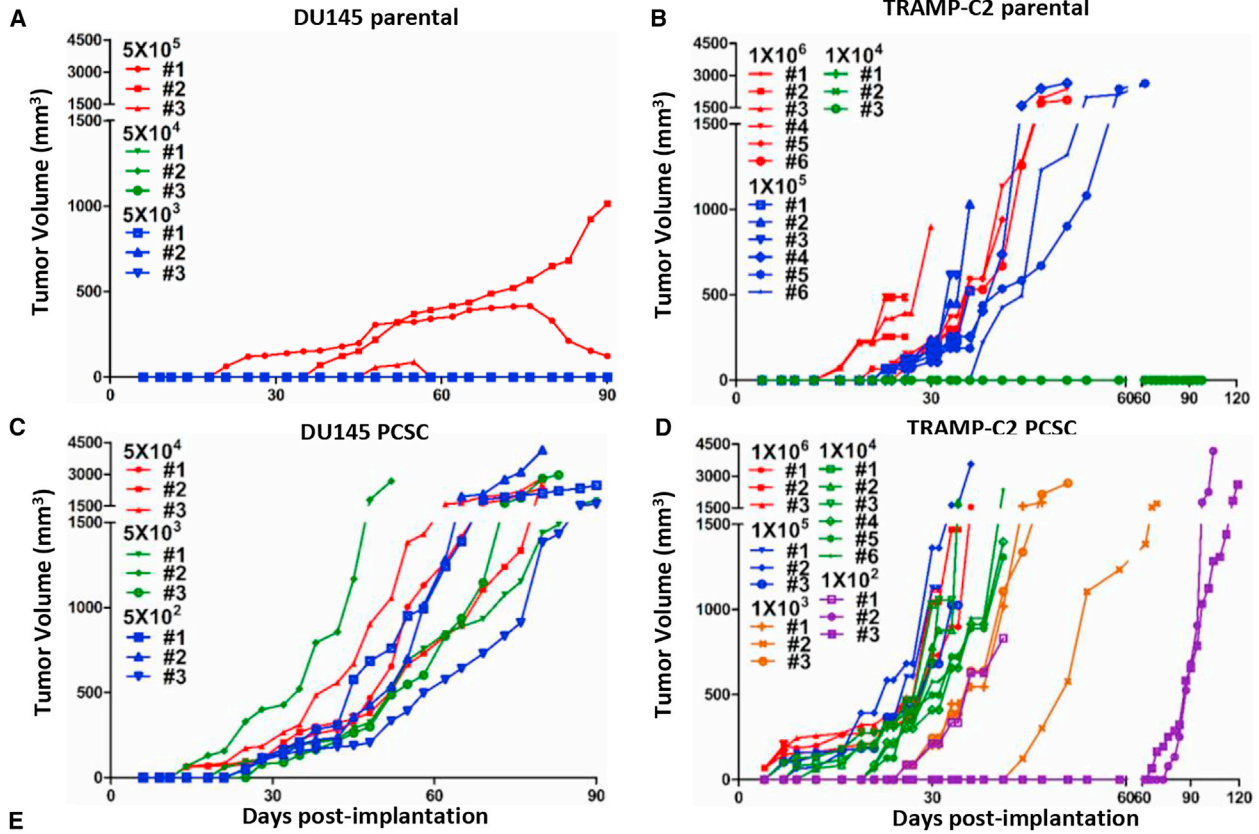
DISCUSSION

There is increasing evidence that initiation, growth, recurrence, and metastasis of cancers are related to the behavior of cancer stem-like cells, including in PCa.^{3,5} Putative stem-like cells have been isolated from PCa biopsies and the DU145 cell line.^{4,20,21} Serum-free medium can enrich the growth of cancer stem-like cells, and the sphere formation assay is a classic method for the analysis of their self-renewal ability.³ We used EF20 medium, commonly used for neural stem cells, to enrich PCSCs from human DU145 and mouse TRAMP-C2 cell lines. Both demonstrated high self-renewal potential and the ability to form spheres from even 1 cell/well. PCSC tumors formed with as few as 5×10^2 DU145 PCSCs and 1×10^2 TRAMP-C2 PCSCs, in contrast to the parental serum-cultured cells that required at least 1×10^5 cells. Both DU145 and TRAMP-C2 PCSCs demonstrated increased resistance to radiotherapy. Thus, we believe that PCSCs provide a better model to test therapeutic strategies.

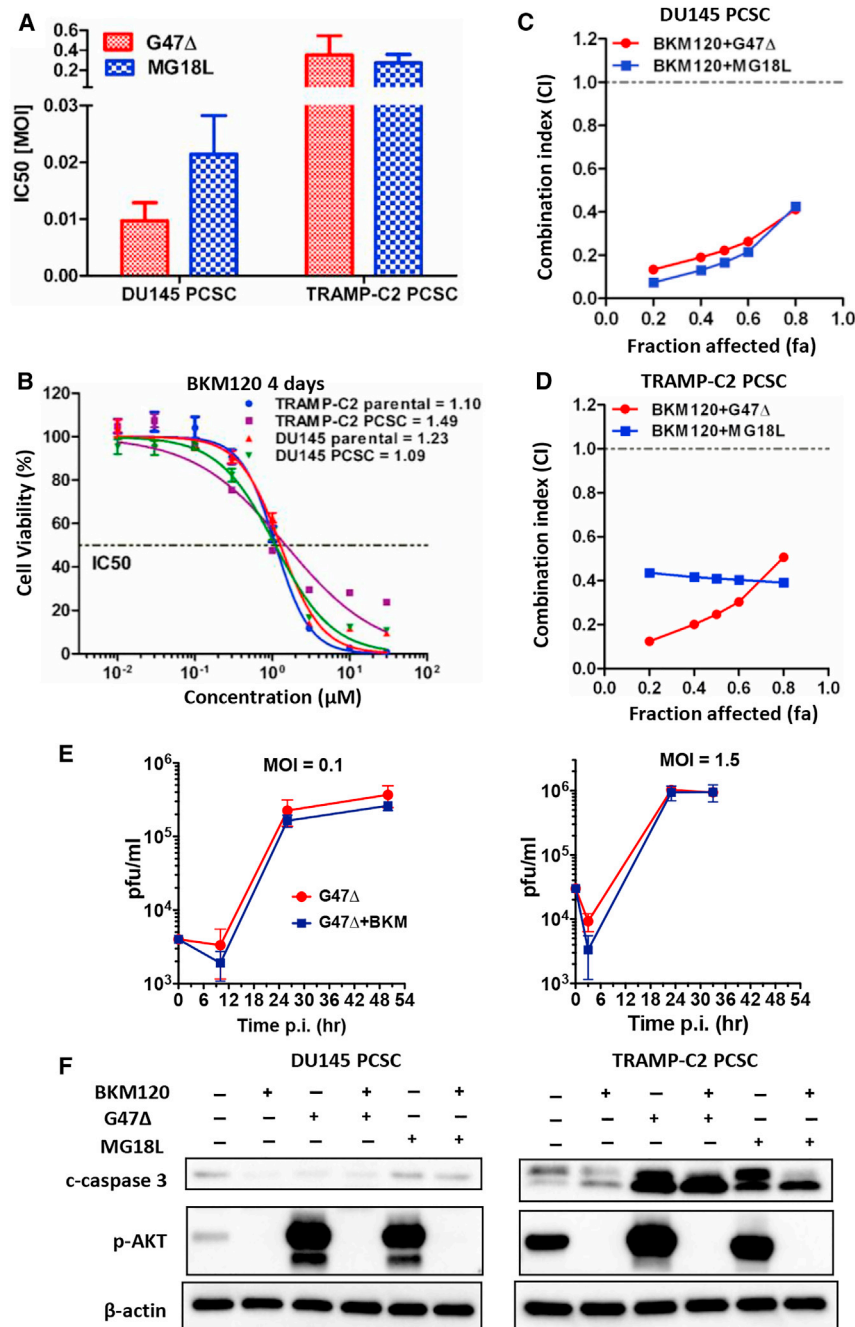
oHSV kills tumors by oncolytic mechanisms, which are different from standard anticancer therapies. In previous studies, we and others demonstrated that oHSV was effective in human PCa cell lines,¹⁰ organotypic cultures,¹⁸ and xenografts;^{14,22,23} in mouse PCa subcutaneous, orthotopic, and metastatic tumors;^{19,24,25} and in spontaneously arising tumors in transgenic mice.²⁶ Here we found that PCSCs *in vitro* were very sensitive to two oHSVs with different genetic alterations, MG18L and G47 Δ . G47 Δ recently completed a phase I clinical trial in Japan for castration-resistant PCa, where it was well tolerated without attributable serious adverse events.⁷ oHSV talimogene laherparepvec (T-Vec), similar

to G47 Δ except expressing GM-CSF, was recently approved by the U.S. Food and Drug Administration (FDA) and European Medicines Agency (EMA) for the treatment of advanced melanoma.⁶

oHSV interacts beneficially with many pharmacological agents in killing cancer cells *in vitro*,¹³ including PCa.^{11,12} Therefore, we tested the combination of chemotherapy, radiotherapy, and oncogenic pathway inhibitors with oHSV to kill PCSCs. Only BKM120 synergized with oHSV. BKM120 neither increased nor decreased the replication ability of G47 Δ . However, it did block the phosphorylated protein kinase B (p-AKT) increase induced by the virus. Additional studies into its mechanism are needed and should include isoform-specific studies, as BKM120 is an oral pan-class I PI3K inhibitor that targets all four catalytic isoforms (p110 α , β , δ , and γ).¹⁶ PI3K-signaling pathways are critical for PCa stem-like cell maintenance, and targeting PI3K signaling may be beneficial in PCa treatment by eliminating PCSCs.²⁷ Targeting PI3K with BKM120 inhibited PI3K signaling in the PCSCs, and it was reported to have anti-proliferative, pro-apoptotic, and anti-angiogenic effects.¹⁵ The combination of a single intra-tumoral G47 Δ injection and systemic BKM120 was effective in inhibiting DU145 PCSC-derived tumor growth and inducing complete regression in some tumors. Treatment-related serious adverse events of BKM120 in prostate clinical trials included fatigue, hyperglycemia, anemia, diarrhea, weight loss, and rash.^{16,28} We noted no skin changes, weight losses, or organ histopathology in our xenograft study. Thus, oHSV combined with BKM120 was not only effective but safe and well tolerated. While two recent clinical trials of BKM120 in metastatic castration-resistant PCa in combination with AR inhibitors did not find any clinical benefit,^{16,28} the synergy with oHSV suggests that this combination warrants clinical evaluation.



(legend on next page)



MATERIALS AND METHODS

Cells, Viruses, and Reagents

DU145 cells (ATCC) were grown in Eagle’s minimum essential medium with 1.5 g/L sodium bicarbonate, non-essential amino acids,

amphotericin B complex (Mediatech). For passaging, spheres were centrifuged, treated with Accutase (Innovative Cell Technologies) at 37°C for 5 min, dissociated by pipetting, washed with PBS, and resuspended in EF20 medium.

Figure 4. oHSV and BKM120 Synergize in Killing PCSCs In Vitro

(A) IC₅₀ values for oHSV (G47Δ or MG18L) in DU145 or TRAMP-C2 PCSCs, from 3 experiments. Mean ± SD. (B) BKM120 dose-response curves for DU145 and TRAMP-C2 PCSCs and parental cells, with IC₅₀ values listed. (C and D) Chou-Talalay synergy analysis for oHSV combined with BKM120 in DU145 (C) and TRAMP-C2 PCSCs (D). Combination index (CI) < 1, CI = 1, and CI > 1 indicate synergistic, additive, and antagonistic, respectively. (E) G47Δ virus (left, MOI = 0.1; right, MOI = 1.5) growth curve in DU145 PCSCs with or without BKM120 (0.2 μM). (F) Representative western blot of BKM120- and oHSV-treated DU145 (left) and TRAMP-C2 (right) PCSCs. Experimental conditions were as follows: BKM120, 10 μM; G47Δ, 1 MOI; and MG18L, 1 MOI. All assays were in the absence of heparin.

L-glutamine, and sodium pyruvate supplemented with 10% fetal bovine serum (FBS). TRAMP-C2 cells, obtained from N. Greenberg (Fred Hutchinson Cancer Research Center, Seattle WA), were cultured in DMEM with 4.5 g/L glucose supplemented with 5% FBS, 5% Nu-serum IV, 10⁻⁸ mol/L dihydrotestosterone, 5 μg/mL insulin, and 25 μg/mL penicillin-streptomycin. Cells were cultured at 37°C and 5% CO₂. Construction of G47Δ, G47Δ-mCherry, and MG18L has been described.^{8,9,29} Compounds used in this study were as follows: Docetaxel Injection (Sandoz), BKM120 (Selleck, dissolved in DMSO), Wnt pathway inhibitor ICG-001 (MedChem Express, dissolved in DMSO), and GSI (PF-03084014, Selleck, dissolved in DMSO).

PCSCs

PCSCs were cultured in EF20 medium composed of Neurobasal medium (Invitrogen) supplemented with 3 mM L-Glutamine (Mediatech), 1 × B27 supplement (Invitrogen), 0.5 × N2 supplement (Invitrogen), 2 μg/mL heparin (Sigma), 20 ng/mL human EGF (R&D Systems), 20 ng/mL human FGF-2 (PeproTech), and 0.5 × penicillin G-streptomycin sulfate-

Figure 3. PCSCs Are More Tumorigenic In Vivo

Tumor growth after subcutaneous implantation of DU145 parental cells (A), TRAMP-C2 parental cells (B), DU145 PCSCs (C), and TRAMP-C2 PCSCs (D). Number of cells implanted and mouse number are indicated. DU145 subcutaneous tumors (E) and TRAMP-C2 subcutaneous tumors (F) stained with H&E (left). Immunofluorescent staining of Ki67 (red) and DAPI (blue) (middle) and quantitation of Ki67 expression (right) are also shown. n = 3, mean ± SD, *p < 0.05. Scale bars, 100 μm.

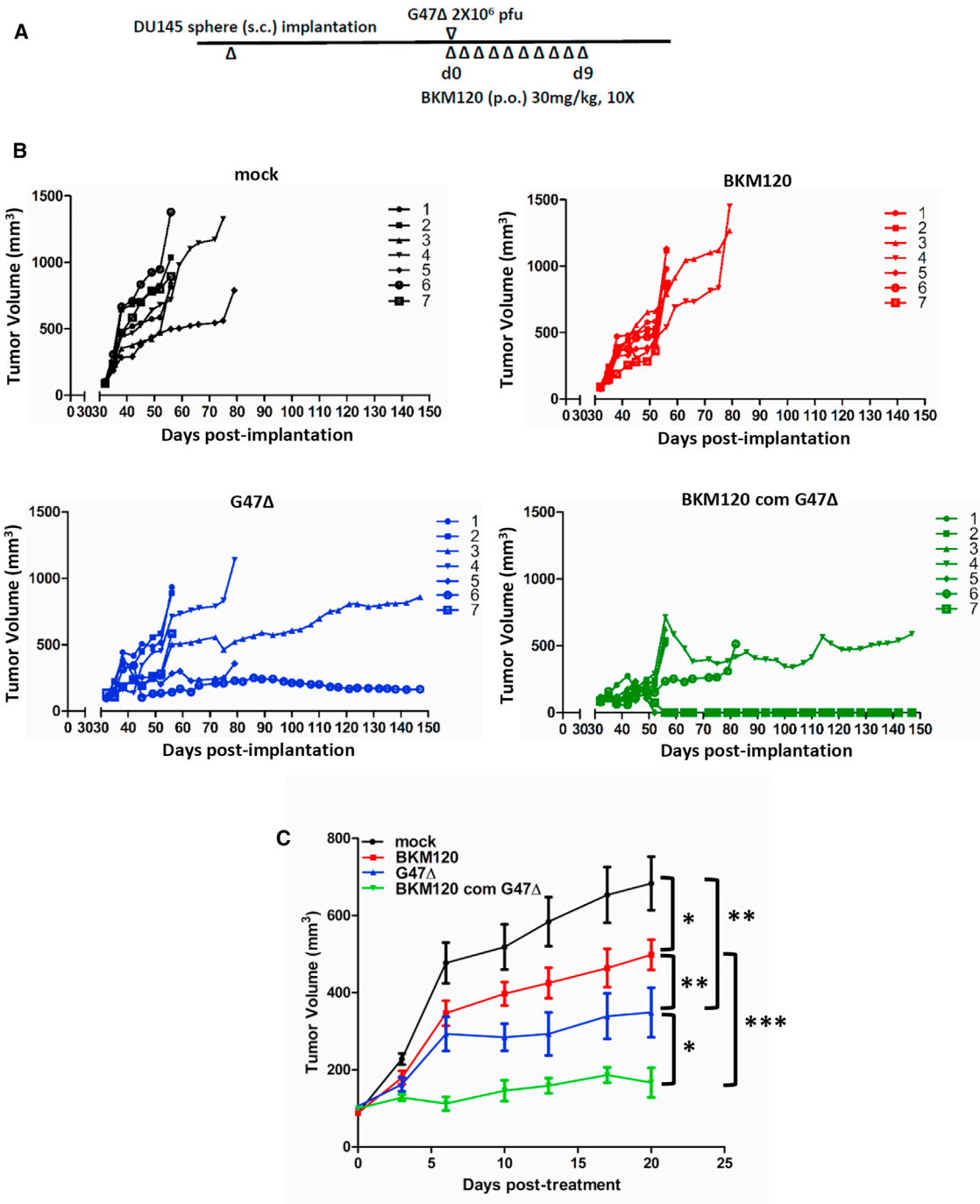


Figure 5. oHSV and BKM120 Combination Therapy In Vivo
 (A) Schematic illustrating the dosing and scheduling of treatment regimens in mice bearing subcutaneous DU145 PCSC-derived tumors. (B) Tumor volumes indicated for individual mice from each treatment group. (C) Mean DU145 PCSC tumor growth of 4 treatment groups \pm SEM from (B). Comparisons between the indicated groups from days 13–20 (except between mock and BKM120, only on day 20); *p < 0.05, **p < 0.005, ***p < 0.0001.

Cell Susceptibility Assays and Chou-Talalay Analysis
 DU145 and TRAMP-C2 parental cells were seeded into 96-well plates at 2,000 and 1,500 cells/well, respectively. DU145 and TRAMP-C2

PCSCs were seeded into 96-well plates at 5,000 cells/well for the 4-day assay or 3,000 and 2,500 cells/well, respectively, for the 6-day assay. Cells were treated with radiation (2, 4, 6, 10, and 20 Gy) or

docetaxel (0.001, 0.01, 0.1, 1, 10, and 100 nM) *in vitro*. G47Δ at an MOI and the indicated compound concentrations were added alone or in combination. 5-(3-Carboxymethoxyphenyl)-2-(4,5-dimethylthiazoly)-3-(4-sulfofenyl)tetrazolium, inner salt (MTS) assays (Promega) were performed according to the manufacturer's instructions, and dose-response curves were plotted (non-linear fit; GraphPad Prism). For Chou-Talalay analysis, experiments were carried out as described.³⁰ Combination index (CI) < 1, CI = 1, and CI > 1 indicate synergistic, additive, and antagonistic interactions, respectively. All experiments were repeated at least three times.

Virus Yield Assay

DU145 PCSCs were dissociated and the following day infected with G47Δ (MOI = 0.1 or 1.5) for 30 min to 1 h. For high MOI, virus inoculum was removed and vehicle or BKM120 was added to 0.2 μM. Infected cells were incubated for the indicated times, and cells and media were harvested (in triplicate), freeze-thawed and sonicated, and titered on Vero cells.

Sphere Formation Assay

Cells were dissociated and passed through a 70-μm filter to produce single-cell suspensions, counted, and diluted to the indicated cell number in 100 μL media/well in a 96-well plate. Then 14 days later, spheres with diameters ≥ 50 μm in each well were microscopically counted.

Immunoblots

Cells (500,000/well) were treated as indicated. Cell pellets were lysed 24 h later in radioimmunoprecipitation assay (RIPA) buffer (Boston Bioproducts, Worcester, MA) plus a cocktail of protease and phosphatase inhibitors (Roche). 18 μg protein was separated by 10% SDS-PAGE and transferred to polyvinylidene fluoride (PVDF) membranes by electroblotting. After blocking with 5% nonfat milk in TBS-Tween 20, membranes were incubated at 4°C overnight with the following antibodies: anti-p-AKT (Ser 473), anti-cleaved-caspase-3, and β-actin (Cell Signaling Technology, Danvers, MA).

In Vivo Studies

For xenograft tumorigenicity studies, DU145 parental and PCSCs were implanted subcutaneously in 6- to 7-week-old athymic male mice (NCI). TRAMP-C2 parental and PCSCs were implanted subcutaneously in 6- to 7-week-old male C57BL/6 mice (NCI). Tumor volume (mm³) = a² × b × 0.52, where a and b are the shortest and longest diameters, respectively. Tumors measuring at least 5 mm in diameter were considered a positive take. For efficacy studies, DU145 PCSCs (5 × 10⁴ in 100 μL) were subcutaneously implanted in male athymic mice. On day 32, randomly grouped mice (N = 7/group) were intra-tumorally injected with G47Δ (2 × 10⁶ plaque-forming units [PFU]) or virus buffer (PBS with 10% glycerol), and/or BKM120 was initiated (30 mg/kg/day, gavage, dissolved in 0.5% methylcellulose, daily for 10 days). Tumor specimens and organs were harvested when tumors reached 15-mm diameter, fixed in 4% paraformaldehyde, and embedded in paraffin. Sections were stained with H&E. All *in vivo* procedures were approved by the

Institutional Animal Care and Use Committee at Massachusetts General Hospital.

Statistics

Unpaired Student's t test (two tailed) was used to analyze significance between two treatment groups. p values less than 0.05 were considered statistically significant (GraphPad Prism 5). For ELDA, data were uploaded into <http://bioinf.wehi.edu.au/software/elda/> and results plotted and analyzed.

SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at <https://doi.org/10.1016/j.omto.2019.03.008>.

AUTHOR CONTRIBUTIONS

L.W. was involved with the conception and performance of experiments, statistical analysis, and writing the manuscript. J.N. assisted with some of the experiments. H.W. designed and supervised some experiments. S.W. and C.W. reviewed histology and analyzed data. M.R.H. performed virus yield assays and assisted with animal studies. S.D.R. and R.L.M. were involved with the conception and design of experiments, supervised, analyzed data, and participated in manuscript preparation. All authors reviewed and edited the manuscript.

CONFLICTS OF INTEREST

The authors declare no competing interests. R.L.M. and S.D.R. are co-inventors on patents relating to oHSV, owned and managed by Georgetown University and Massachusetts General Hospital, for which royalties have been received.

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