HHS Public Access

Author manuscript

J Biomed Nanotechnol. Author manuscript; available in PMC 2017 October 24.

Published in final edited form as:

J Biomed Nanotechnol. 2015 February ; 11(2): 274–281.

Radiosensitization of Hs-766T Pancreatic Tumor Xenografts in Mice Dosed with Dodecafluoropentane Nano-Emulsion–Preliminary Findings

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Abstract

Tumor hypoxia is an important mediator of radiation therapy resistance. We conducted a study to investigate whether an oxygen therapeutic based upon dodecafluoropentane (DDFP) nano-emulsion (NVX-108) could increase tumor pO_2 in hypoxic tumors and improve radiation response. Pancreatic (Hs-766T) tumor xenografts were grown in the flanks of 29 SCID mice. Direct tumor pO_2 measurements were performed in 9 mice treated with 0.3, 0.45 and 0.6 cc/kg NVX-108 (2% w/vol DDFP) in order to assess the dose dependent increase in tumor pO_2. Twenty mice were randomized into 3 groups including control (no treatment), carbogen breathing treated with 12 Gy radiation, and carbogen breathing treated with 12 Gy radiation and NVX-108 (0.6 cc/kg NVX-108 administered as 30 minute IV infusion at time of radiation). Tumor volume was monitored to assess treatment efficacy. Results showed that tumor pO_2 increased in NVX-108 treated mice up to 400% with the greatest effect seen at the highest dose of 0.6 cc/kg. Tumor growth was significantly reduced in both treatment groups relative to controls ($p < 0.0001$). The combination of carbogen, radiation, and NVX-108 demonstrated a 2-fold reduction in average tumor volume compared to carbogen plus radiation treatment ($p = 0.01$). Further study of NVX-108 as a radiation sensitizer is warranted.

Keywords

Tumor Hypoxia; Radiation; Oxygen Therapeutic; Dodecafluoropentane; Nano-Emulsion; Radioensitization

INTRODUCTION

Tumor hypoxia is an important factor limiting response to radiation treatment.[1–3] Hypoxic tumors require about three-fold higher radiation dose than normoxic tumors for comparable effect on tumor cells.[1] About two-thirds of all solid tumors are hypoxic.[4–6] Hypoxia is predominant in tumors such as pancreas, head and neck, lung and high-grade brain

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neoplasms.[7] In brain tumors hypoxia is associated with poor survival.[8] Normal tissues have interstitial pO_2 values of about 25 mm of Hg (or greater) but cancers pO_2 values commonly range from 2 mm Hg (pancreas) to 14 mm Hg (sarcoma).[7] Radiation sensitivity drops precipitously below a critical oxygen sensitivity of about 25–30 mm Hg; above that level, e.g., super-oxygenation, does not generally increase risk to normal tissues.[5–7]

Different strategies have been pursued to increase tumor oxygenation as adjunct to radiation therapy (RT). These strategies include hyperbaric oxygen[9] allosteric hemoglobin (Hgb) modifiers,[10] modified hemoglobin derivatives and perfluorocarbon emulsions (FCe).[11–13] Almost all of these strategies have failed due to high doses and resultant adverse events (FCe)[11] and side effects (Hgb).[14] Despite some evidence of efficacy,[15] hyperbaric oxygen is seldom used in radiotherapy. Most recently trans sodium crocetinate, an agent that reportedly increases oxygen diffusion, finished a Phase II clinical trial as a radiation sensitizer in treatment of glioblastoma with concomitant RT and chemotherapy.[16] The data from this study has not yet been released.

It has been predicted that microbubbles should carry far more oxygen than liquids.[17,18] NVX-108 is an oxygen therapeutic (OT) composed of emulsified dodecafluoropentane which is a unique fluorocarbon material with quasi-bubble properties.[19] These properties may increase its oxygen carrying capacity.[19] The material was previously shown to reverse radiation resistance in a hypoxic tumor model,[20] but the effect was demonstrated *ex vivo* and tumor pO_2 studies were not performed. This study was performed to see if NVX-108 administration would increase tumor pO_2 and improve response to radiation *in vivo* in a hypoxic tumor model.

Radiation and chemotherapy are commonly used to treat pancreatic cancer.[21] Hs-766T pancreatic cancer is an aggressive form of pancreatic cancer that is generally resistant to drug therapy and radiation and Hs-766T tumor xenografts have been shown to be hypoxic.[22–25] Accordingly, the Hs-766T tumor model was chosen for this study.

METHODS

NVX-108 Product

Preparation of NVX-108 (Lot 080611)—Dodecafluoropentane ((DDFP) FluoroMed, Round Rock, TX) was emulsified at 2% (w/v) in a 30% (w/v) sucrose solution, buffered at physiological pH (~ 7). A custom purified medical grade form of fluorosurfactant, PEG Telomer B ((PTB) J. Tech Sales, Boca Raton, Fla.), was used at a 0.3% (w/v) concentration in combination with an Emulsiflex C-5 homogenizer (Avestin, Ontario, Canada) to reduce and stabilize the particle size at ~ 250 nm. Because of the volatility of DDFP (BP = 29 °C), stainless steel (316L) pressure vessels (PVs) with jackets for chilling were designed and fabricated for controlling the temperature and the pressurization of the product during the 3 phases of the compounding procedure. All process flow paths between PVs were staged with 1/4 inch I.D. flexible nylon tubing. The ends of the tubing lines were fitted with 1/2 inch tri-clamp fittings which were chosen to smoothly and aseptically connect flow paths between the homogenizer and the PVs. Thus, the product was fully shielded from the room

environment during the entire manufacturing process. The process temperature was controlled at 4 °C and the pressure was controlled from 5–7 psi using compressed nitrogen.

The emulsion was recirculated through the homogenizing valve for 6 passes (effectively) at 14,000 psi and then immediately filtered through a 0.2 μm sterile filtration capsule. Using a Unispence (Wheaton, Millville, NJ) filling machine, the resulting product was filled into 5 mL vials which were promptly stoppered by hand and crimped with a pneumatic Power Crimper (Kebby Industries).

Characterization

Particle Sizing—Emulsion particle size was assessed in triplicate at 0, 1, 2, 3 weeks and at 1, 2, 4, 6 and 11 months. At each time-point, 3 vials were selected at random and analyzed by dynamic light scattering (Nicomp 380, Particle Sizing Systems, Fort Richey, Fla.). In order to minimize the effect of gradual temperature increase during size determinations, the sample temperature was controlled at 19 °C.

DDFP Concentration—DDFP concentration was measured in quintuplicate at time points of 0, 3, and 6 months. At each time point, 5 vials were randomly selected and assayed by GC headspace analysis with FID detection. Sample concentrations were established by customary comparison to linear standard curves having R^2 values >0.98.

Hydrogen Ion Concentration (pH)—The pH of the final product was measured in quintuplicate at time-points of 0, 3 and 6 months. At each time-point, 5 vials were randomly selected and directly measured for hydrogen ion concentration using a hydrogen ion selective probe. At each time-point, calibration standards were run at pH values of 4, 7 and 10.

Sterility and Endotoxin Testing—Thirteen vials of the finished product were randomly selected from various places throughout the batch fill order. They were shipped to Charles River Labs (Malvern, PA) for sterility analysis (USP 71, membrane filtration) and determination of endotoxin levels (USP 85, Limulus Amebocyte Lysate, kinetic chromogenic assay). Three of the vials were each tested separately for endotoxin levels (n = 3) and the other 10 vials were pooled together for a single sterility test of the batch (n = 1).

Cell Culture

The Hs-766T (pancreatic; ATCC Cat #HTB-134) cell line utilized for this study was obtained from the American Type Culture Collection (ATCC, Manassas, VA) and was handled, stored and managed by the University of Arizona Experimental Mouse Shared Services (EMSS, University of Arizona). Cells were grown in DMEM (Mediatech) with high glucose, L-glutamine and 10% fetal bovine serum and maintained at 37 °C with 5% CO_2. Tumor cells were authenticated through ATCC Cell Authentication testing services by way of PCR/short tandem repeat (STR) profiling. Cells were routinely tested for mycoplasma using the Universal Mycoplasma Detection Kit (ATCC, 30-1012K), and found to be free of contamination.

Tumor Hypoxia Measurements and Radiosensitization

Animal Model—Twenty-nine female, SCID mice between the ages of 5–8 weeks of age were used in these studies. All mouse feeding, husbandry and veterinary was managed by the EMSS under IACUC approved guidelines and protocols. Mice were caged in groups of four or less, and fed and watered *ad libitum*. Prior to injection, mice were shaved to ascertain a suitable site for the tumor xenografts. Tumor cells (10×10^6 cells in Matrigel™; BD Bioscience) were injected subcutaneously on the left rear flank of each mouse. Tumor burden evaluations were made twice weekly using electronic calipers to determine tumor size ($(a^2 \times b^2)/2$). When tumors reached a mean volume of 500–700 mm^3 the mice were randomized to one of 6 groups: 3 groups of mice for tumor O_2 measurements and 3 groups of mice for tumor growth measurements.

Tumor pO_2 measurements were performed in 9 of the mice. NVX-108 doses of 0.3 (2 mice), 0.45 (3 mice) and 0.6 (4 mice) mL/kg were administered.

Tumor growth rate was studied in the other 20 mice. These mice were designated to the following 3 treatment groups: Group 1: No treatment (4 mice), Group 2: breathing carbogen while being treated with a 12 Gy radiation dose (8 mice), Group 3: Treated with NVX-108 (0.6 cc per kg, 2% w/vol dodecafluoropentane (DDFP) administered IV over 30 minutes with radiation at end of infusion) and breathing carbogen while being treated with a 12 Gy radiation dose (8 mice).

Radiosensitization—Each mouse received ketamine (65 mg/kg IP), xylazine (13 mg/kg IP) and acepromazine (2 mg/kg IP) for immobilization and was fitted with a tail-vein catheter for dosing by tail vein injection (TVI). The tail-vein catheterization was achieved by using a fabricated 27-gauge needle catheter retrofitted to PE 50 tubing. The catheter was firmly affixed to each animal's tail using 3–0 suture thread and specialized adhesive tape on both sides of the tail. Following catheterization, the mice were placed within a custom-built gas chamber introducing carbogen which was ventilated at the 10-minute mark. They were held in custom restraints in order to be positioned under a lead shield isolating the flank tumor xenograft for radiation fractionation. A cobalt-60 teletherapy unit (Atomic Energy of Canada Limited Theratron-80) was used to irradiate the mice. Cobalt-60, decays to Nickel-60, ($^{60}Ni_{28}$) by the emission of beta particle. The activated nickel nucleus emits two gamma ray photons with energies of 1.17 MeV and 1.33 MeV resulting in an average beam energy of 1.25 MeV.[26–29] The energy of these gamma rays is used to treat conditions like cancer.[29] All mice underwent a single fractionation of 12 Gy to the tumor within a custom-built gas chamber introducing carbogen (95% O_2, 5% CO_2) which was ventilated at the 10-minute mark. The calculation for the XRT dose duration was based on "prescribed dose/dose rate" = (1200 cGy)/(87.9 cGy/min) amounts to a 13.65 minute (13 m 39 s) single fractionation on the tumor. For group #2,200 μL sterile saline was introduced via TVI (Time 0:00) and served as the sham injection commencing 10 minutes prior to carbogen breathing and 16.35 minutes (16 m 21 s) before irradiation. For group #3, 200 μL of NVX-108 was injected by way of TVI (Time 0:00) 10 minutes before carbogen breathing and 16.35 minutes before irradiation. Tail vein injections were performed with a multi-syringe pump for simultaneous administration to each group. Simultaneous injections of NVX-108 and

saline initiated the study at time 0:00 minutes. This was followed by carbogen breathing at 10 minutes and then radiation at 16.35 minutes.

Once radiation was completed, the mice were allowed to recovery and have food and water ad libitum. Tumor size bi-dimensional measurements were performed twice per week. When tumor size was greater than 1 gram, mice were sacrificed.

Measurements of Tumor Hypoxia—Tumor oxygenation and blood flow levels were monitored using OxyLab (Oxford Optronics, Oxford, UK) triple parameter E-series fiber-optic probes stereotactically inserted into all tumors. Oxygen partial pressure signals from these monitors were recorded in real-time using a multi-channel data acquisition system (Power-Lab 8SP, ADInstruments, Australia) running under Chart™ for Windows™ (Ver.5.02, ADInstruments, Australia). Mice were initially anesthetized with 4–5% Isoflurane® 95–96% O_2 and then once immobilized were switched to 1.5–2.0% Isoflurane and 98–98.5% carbogen. The mice were restrained on a custom immobilization platform to prevent movement and retrofitted with a heating pad to sustain body core temperature. Precaution was taken to prevent any movement of the hypoxia probes and eliminate interference from external light sources to prevent probe artifact. Tumors were penetrated using a 19 gauge needle to a depth of ~ 2–4 mm and microprobes (OD ~ 450 μm) were fed through the needle into the tumor xenografts and fixed in position using stereotactic methods. Microprobes were carefully marked with gradations in order to reach the same depth in all tumors. Once the probes were stabilized and immobilized the output signals were monitored (5–10 min) until a stable baseline was observed. Real-time measurements were taken for 10 minutes at baseline on carbogen and following a 200 μL IV injection via tail vein of doses of 0.3, 0.45 or 0.6 cc/kg NVX-108 (NuvOx Pharma Tucson, Arizona) while animals continued to breathe carbogen.

Data Analysis

Pharmacokinetic data of oxygen levels in tumors were analyzed using PK Solutions software (version 2.0.6, Summit Research Services, Montrose, CO, USA). Multi-compartmental modeling was employed in the PK parameter estimations. All statistical analyses were carried out with Unistat® Statistical Software Package Version 6.0.09 (Unistat Ltd., London England). In addition to descriptive statistics (basic mean and standard deviation values) for tumor oxygen levels and tumor growth, simple regression curves/equations were calculated for each of the 3 tumor growth groups. Two main comparisons were made: (1) The significance of the slope of each regression line was obtained to determine whether it was truly different from “zero” (the definition of no change with time). A resulting slope that is not significantly different ($p > 0.05$) from “zero” indicates the treatment prevents or reverses tumor growth. (2) The mean and standard deviation slope values of the regression lines were compared to each other for significance of difference. There were 3 comparisons made: Group 1/Group 2, Group 1/Group 3 and Group 2/ Group 3. A resulting p-value less than 0.05 indicates that the slopes of 2 lines are significantly different from one another.

RESULTS

NVX-108 Product

Characterization—Certain parameters of the NVX-108 lot used in the mouse studies (particle size, DDFP concentration and pH) were monitored over the course of the study to rule out potentially confounding effects should the product fall out of specification during the study. Sterility and endotoxin load were only tested at the start of the study, as once sterility is established it is theoretically permanent and consequently endotoxin levels (residual lipopolysaccharides from the cell membranes of gram negative bacteria) cannot increase.

Particle Sizing—Figure 1 shows the data for average ± standard deviation of particle diameter for 11 months following batch production. The average particle size did not exceed 260 nm. Furthermore, 99% of the particles were measured to have diameters ≤ 400 nm throughout the study. Thus, it is reasonable to hypothesize that NVX-108 particles may remain small enough in the vasculature to pass through leaky (more porous) membranes, facilitating delivery of oxygen to hypoxic tissue.

DDFP Concentration—At time zero the DDFP concentration of NVX-108 lot 080611 was measured to be 2.2±0.0% (w/v). At 3 month and 6 months it was found to be 2.0 ± 0.5% (w/v), and 1.9 ± 0.5% (w/v), respectively. There was no significant change in DDFP concentration during this experiment.

Hydrogen Ion Concentration (pH)—The pH of the batch remained consistent throughout the experimental period. The average pH of 5 samples taken at each time point did not change over the 6 month period. Furthermore, the pH was maintained within specification at 7.

Sterility and Endotoxin Testing—Table I shows the results of endotoxin testing in the specific lot of NVX-108 used in these experiments. The 10 vials pooled for sterility analysis showed no microbial growth (“0” colony forming units) over the standard 14 day test period (see Table I). As is indicated in Table I, each of the 3 vials tested for endotoxin level showed less than 0.8 EU/mL. The final endotoxin load was determined to be 0.67 ± 0.05 EU/mL. Note that the specification for the final product is ≤ 5 EU/mL based on known intended doses for administration to mice in combination with acceptable endotoxin levels in mice. The final product was determined to be safe for intravenous administration.

Measurements of Tumor Hypoxia—Table II shows PK estimates of oxygenation in Hs-766T tumors. The pharmacokinetic parameter estimates of tumor oxygenation from the 4 mice that received 0.6 mL/kg NVX-108, the 3 mice that received 0.45 mL/kg and the 2 mice that received 0.3 mL/kg NVX-108 were generated using multi-compartmental modeling (MCM). Note that MCM analysis was chosen for 2 reasons. First, DDFP (the ingredient in NVX-108 responsible for oxygen delivery) exhibits multi-compartmental pharmacokinetics as it largely distributes outside of the vascular space.[30] In addition, oxygen is not directly injected into the tumor. Rather it must be picked up in the lungs and carried by the DDFP injected into the vasculature. Thus, in addition to the delay in oxygenation of a tumor due to

the distribution phase of NVX-108 outside of the vasculature, the oxygenation rates in tumors as facilitated by a single bolus administration of NVX-108 are expected to show absorption and elimination phases. For first order drugs, the elimination $t_{1/2}$, t_{max} and V_d are independent of dose while the C_{max} and AUC are dose dependent. In the present case, statistical analysis shows that the AUC significantly increased with dose ($p = 0.03$) but that the increase in C_{max} with dose was less significant ($p = 0.11$). Note that small samples sizes are likely to be responsible for lack of statistical power. However, the trends of these increases in combination with the non-significant differences with dosing for $t_{1/2}$ ($p = 0.18$), t_{max} ($p = 0.65$) and V_d ($p = 0.32$) do suggest first order kinetics.

Figure 2 shows the individual oxygen level and blood perfusion traces for all 9 animals used in the oxygen probe study. The oxygen concentration/time data from these 9 animals were used to calculate the parameters displayed in Table I. The mean±standard deviation values for baseline O_2 and blood perfusion levels in the tumors were 1.45±2.45 mmHg and 95±64 BPU, respectively. In some cases, a change in oxygen level might be explained by a causational change in blood perfusion. For instance, the tumor in mouse 011 showed oxygen level increase and decline that directly corresponded with the concurrent change in blood perfusion. However, mouse 020 and 024 tumors showed notable increase in oxygen levels from 20 minutes to 100 minutes, but the blood perfusion data only show increases starting at 60 minutes. Thus, the oxygen level increase observed as early as 20 minutes cannot be attributed to blood flow change. More remarkably, the oxygen increase observed in the tumor of mouse 015 from 20 minutes through 140 minutes was concurrently associated with a low and unchanging blood perfusion rate (< 50 BPU). This suggests that with NVX-108 administration, tumor oxygen levels can be increased even in areas where blood flow is very limited. In addition, the blood perfusion data for the tumor in mouse 009 suggest oxygen levels would be expected to increase from 60 minutes out to 140 minutes. Contrarily, oxygen levels in the tumor of mouse 009 increased slightly from 20 to 40 minutes and then declined steadily out to 100 minutes.

Note that the elimination phase for oxygen in tumors in mice 015, 020 and 024 are not shown in the graphs. These data were not obtained as the oxygen probes were removed after 155 minutes. The estimated terminal half-life values provided in Table II for these tumors were those automatically calculated by the modeling program software. Therefore, the individual $t_{1/2}$ values estimated for mice 015, 020 and 024 were not used in determining the average values.

Figure 3 shows the results of the radiosensitization experiments. There were significant differences in the rates of tumor growth between all groups (control, carbogen + RT and carbogen + RT + NVX-108), $p < 0.05$. The rate of tumor growth was 25x slower for the carbogen + RT + NVX-108 group than control ($p < .0001$). Tumor growth was twice as slow in the carbogen+RT+NVX-108 group than in the carbogen + RT group, ($p = .01$).

DISCUSSION

It has been predicted that microbubbles should carry far more oxygen than liquids.[17,18] Dodecafluoropentane (DDFP), the fluorocarbon material in NVX-108, has a boiling point of

about 29 °C.[19] Because of intravascular pressure, NVX-108 in the blood stream, stays in the condensed (i.e., liquid state) state, but still displays bubble or near-bubble properties that make it highly effective at carrying oxygen.[19] Other fluorocarbon agents have previously been studied as OTs for radiosensitization.[11–13] The prior agents were much higher molecular weight materials with high boiling points and required high doses. The high doses were associated with adverse events in clinical trials ultimately preventing clinical development of these agents.[11] As an example, in a clinical trial of Fluosol (20% w/vol) in radiation therapy of head and neck cancer, the material was administered at doses of either 8 or 9 cc per kg.[11] Patients could only tolerate administration of the material prior to the first fraction of radiotherapy each week for the first five weeks, not prior to each fraction as would be ideal. Eight out of fifteen patients developed abnormal liver function tests.[11] The dose of fluorocarbon used in that study was 130 to 150 times higher than the dose of fluorocarbon in NVX-108 used in this present study. Generally dose scales with body surface area, so a much lower dose per kg body weight might be expected in a study in humans as opposed to in rats or mice (as used in this study).[30] In another study testing Fluosol as a radiosensitizer in a rat tumor model, the dose of fluorocarbon was 250 times higher than we used for NVX-108 in this study.[31] Comparing animal studies, NVX-108 requires less than about 1/200th dose of the prior fluorocarbon materials.[12,13,31] Dodecafluoropentane emulsion (identical to NVX-108 except for the addition of physiological buffer in NVX-108) was previously studied as a contrast agent in more than 2,000 patients[32,33] and was safe at doses that appear to be effective as OT, e.g., for radiosensitization of hypoxic tumors. Additionally, pharmacokinetic studies of DDFPe in human volunteers have shown a short-half life for the material with rapid clearance of DDFP intact via exhalation from the lungs.[33] Still, as shown in our study the effect on tumor oxygenation lasts a couple of hours which should be long enough to allow for radiosensitization during radiation therapy. The relatively short half-life and rapid clearance could be advantageous for administration of the material during each fraction of radiation therapy. If safety in clinical setting of radiotherapy is confirmed, NVX-108 might potentially be used as a sensitizer with each fraction of radiotherapy.

It appears that NVX-108 is a highly effective OT in tumors increasing tumor pO_2 by 400% in this pancreatic tumor xenograft model. By comparison, in a different tumor model, the agent trans sodium crocetinate (oxygen diffusion enhancer) increased tumor pO_2 by 40%.[34] The oxygen electrode used in our studies provided pO_2 and blood flow data from a single point within the tumor tissue. In the tumors that were most necrotic, the pO_2 was zero in some of the tumors and the zones of the tumors that were entered by the probes were likely avascular. Profoundly necrotic tumor showed little change in pO_2. It would be useful to acquire 3-dimensionsal data on tumor oxygenation. PET scanning has been used with hypoxia binding agents to show reversal of tumor hypoxia.[31] MRI, with BOLD, TOLD and proton MRS (e.g., lactate) may also enable characterization of reversal of tumor hypoxia.[36,37]

Both RT + carbogen and RT + carbogen + NVX-108 groups showed significant reduction in tumor growth compared to control. RT + carbogen + NVX-108 had significantly reduced tumor growth compared to RT + carbogen. We hypothesize that the difference between NVX-108 and the RT comparator group would be even greater at lower doses of radiation.

Very high doses of radiation (e.g., 12 Gy) as used in this study may compensate for the oxygen sensitization effect.[38] Note that radiation is typically fractionated and not usually delivered in single doses as high as 12 Gy. A common protocol for treatment of brain tumor, for example, is to administer 60 Gy in 30 fractions of 2 Gy each over 6 weeks.[39] It was not possible to perform a fractionated study giving multiple lower doses of radiation within the scope of this study.

CONCLUSIONS

NVX-108, nano-emulsion of DDFP, retains stable particle size on long-term storage at room temperature. Compared to higher molecular weight, high boiling point liquid fluorocarbons, DDFPe requires a much lower dose of fluorocarbon for use as an oxygen therapeutic. The material shows promise as a radiation sensitizer. Single bolus doses of 0.6 cc/kg (2% w/vol DDFP) resulted in tumor pO_2 increases up to 400% lasting up to 2 hours and significantly improved survival of radiated animals. Prior experience with this agent as an ultrasound contrast agent indicates that it is well tolerated. Further development of NVX-108 as a radiation sensitizer is warranted.

Acknowledgments

The authors acknowledge support by NCI SBIR, 1R43CA144817-01. The authors also acknowledge the help of Ms. Gillian Paine-Murrieta, Manager, EMSS, AZCC. EMSS is supported by the Arizona Cancer Center's Comprehensive Cancer Center Support Grant.

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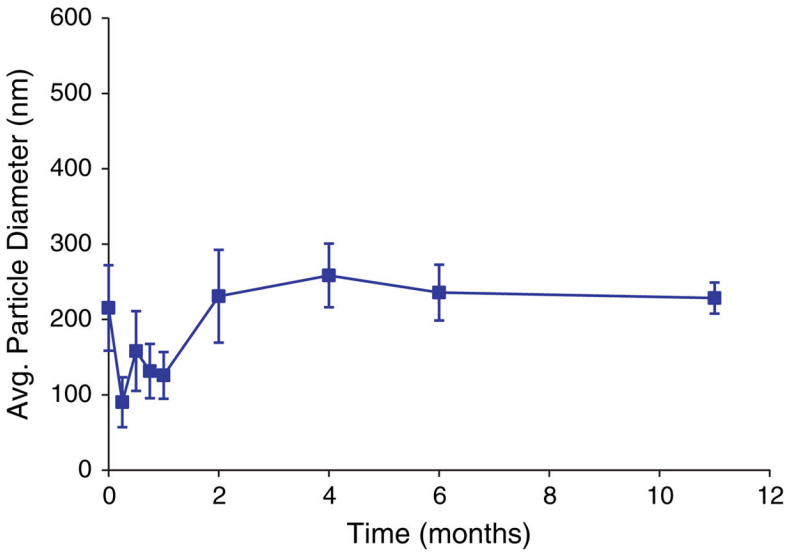

Figure 1.

Particle sizing of NVX-108 lot 080611 over 11 months. The error bars represent one standard deviation of 3 samples at each time point.

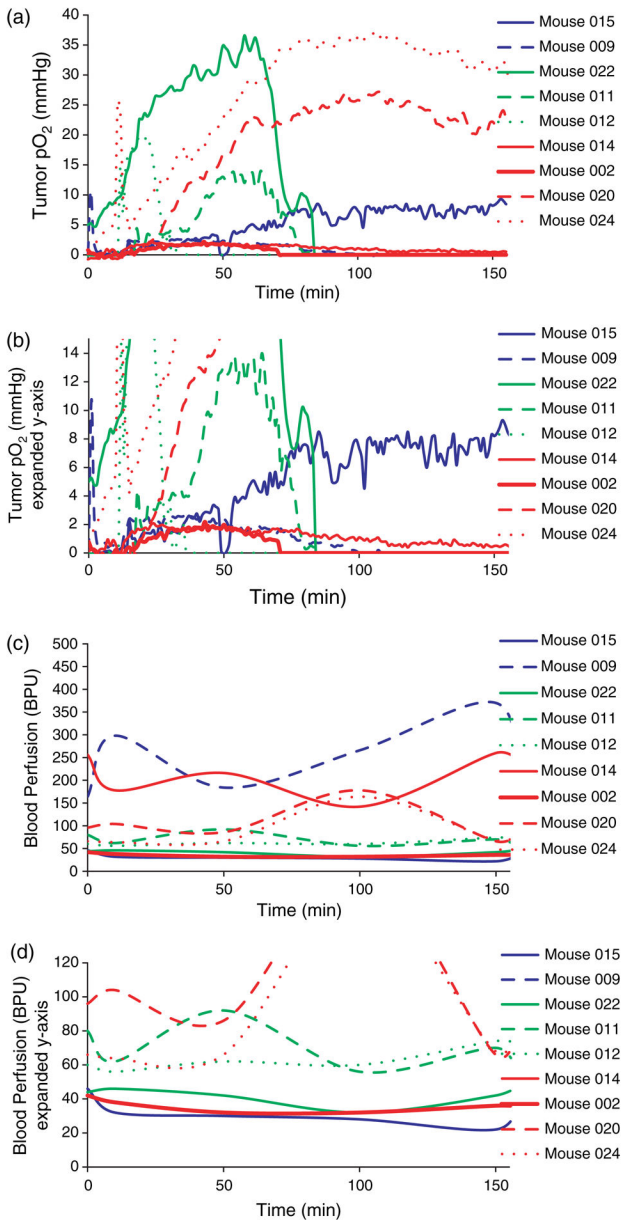

Figure 2.

Oxygen (a) and (b) and blood perfusion levels (c) and (d) in 9 HT766t tumor xenografts in mice. Figures (b) and (d) are expanded views of the *y* -axes from Figures (a) and (c), respectively. They are shown to better view the oxygen and perfusion changes in tumors with lower average values. The different traces (lines) in all graphs represent 9 tumors in 9 different mice. Each mouse was given a single dose of NVX-108: 0.3 mL/kg (blue traces), 0.45 mL/kg (green traces) or 0.6 mL/kg (red traces).

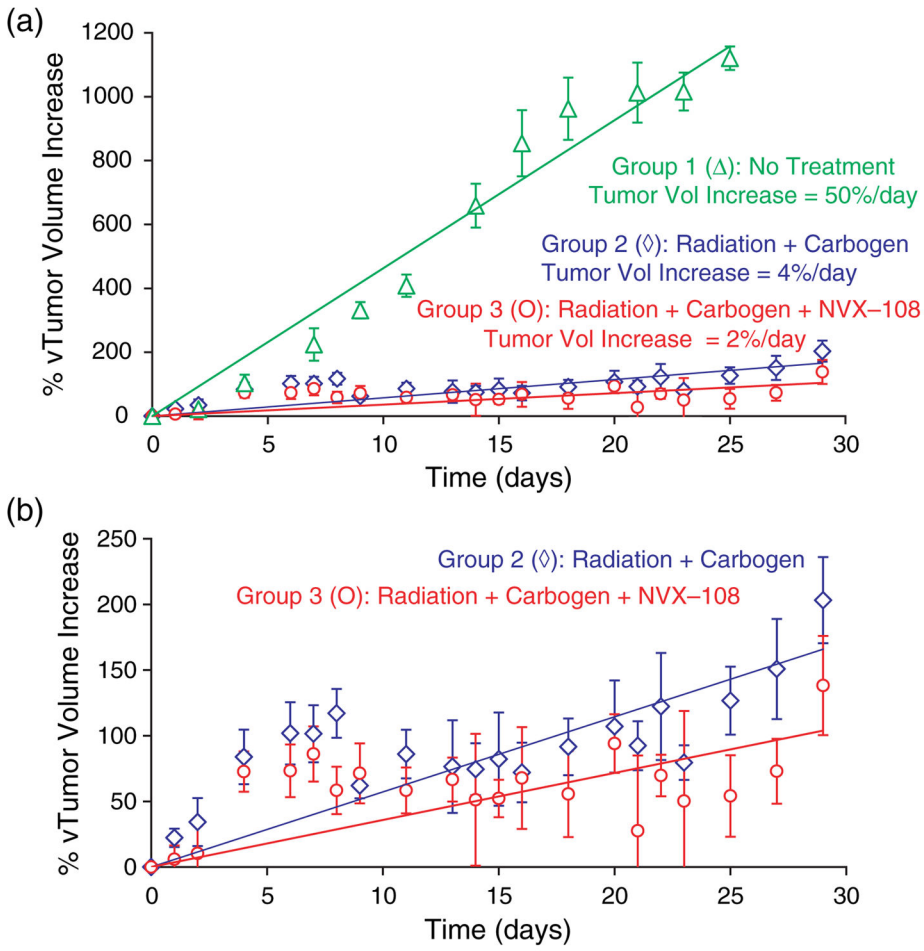

Figure 3.

Comparison of % tumor growth among 3 groups of mice (a) and expanded view of the 2 treated groups (b). Group 1 mice were breathing air and not treated [triangles; $n = 4$]. Group 2 mice were breathing carbogen and their tumors were radiated [diamonds; $n = 8$]. Group 3 mice were breathing carbogen, were dosed with 0.6 mL/kg NVX-108 and their tumors were radiated [circles; $n = 8$]. The slopes of the trend lines are significantly different among all 3 groups ($p < 0.05$). Most notably, the rate of tumor growth (slope) in radiated mice treated with NVX-108 was 25 times slower compared to untreated mice ($p < 0.0001$) and 2 times slower compared to radiated mice ($p = 0.01$).

Table I

Endotoxin levels in NVX-108 Lot 080611 as determined by LAL testing.

Sample vial number	Endotoxin level (EU/mL)
Vial 1	< 0.63
Vial 2	< 0.66
Vial 3	< 0.73
Mean	< 0.67
SD	0.05

Table II

Pharmacokinetic parameter estimates of O_2 levels in Hs-766T tumors in mice dosed with NVX-108. Note these values have been normalized to tumor volume size (mm^3).

NVX-108 dose mL/kg	Mouse ID	$t_{1/2}$ elim. min/mm^3	T_{max} min/mm^3	C_{max} mmHg/mm^3	AUC mmHg × min/mm^3)	V_d mL/kg · mm^3	Tumor volume mm^3	Avg. blood perfusion BPU
0.3	015	0.021[a]	0.14	0.007	0.84	0.09	1307.5	40 ± 18
	009	0.017	0.03	0.003	0.17	0.58	863.6	236 ± 68
0.45	022	0.007	0.07	0.047	2.37	0.03	775.8	50 ± 9
	011	0.003	0.06	0.013	0.44	0.06	1088.3	80 ± 38
	012	0.002	0.02	0.017	0.22	0.07	1155.1	68 ± 18
0.6	014	0.056	0.03	0.002[b]	0.20[b]	2.84[b]	898.5	285 ± 201
	002	0.020	0.05	0.003[b]	0.09[b]	2.28[b]	800.2	59 ± 103
	020	0.098[a]	0.19	0.054	9.02	0.17	503.4	92 ± 26
	024	0.034[a]	0.11	0.037	5.05	0.06	992.8	127 ± 89
MEAN		0.02	0.08	0.005[c,g]	0.501[c,f]	0.15	931.7	
				0.026[d,g]	1.010[d,f]			
				0.046[e,g]	7.033[e,f]			

Notes:

[a] values automatically calculated by the modeling software but not used in determining statistics;

[b] values likely to have been caused by large fluctuations in blood perfusion thorough out measurement period (SD higher than 70%). These were not used in determining statistics;

[c] at 0.3 mL/kg dose of NVX-108;

[d] at 0.45 mL/kg dose of NVX-108;

[e] at 0.6 mL/kg dose of NVX-108;

[f] significant difference at $p = 0.03$ (ANOVA);

[g] significant difference at $p = 0.11$ (ANOVA).