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(54) Title: CANCER TREATMENT USING VIRUSES, FLUOROPYRIMIDINES AND CAMPTOTHECINS

(57) Abstract: Mammalian subjects having a neoplasm are treated with a virus, a fluoropyrimidine, for example 5-fluorouracil, and a camptothecin compound. The virus is selected from the group consisting of a Newcastle disease virus, a measles virus, a vesicular stomatitis virus, an influenza virus, a Sindbis virus, a picornavirus, and a myxoma virus.

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CANCER TREATMENT USING VIRUSES, FLUOROPYRIMIDINES AND CAMPTOTHECINS

BACKGROUND OF THE INVENTION

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Coadministration of certain viruses with 5-fluorouracil to treat cancer is disclosed in WO 94/25627 (page 12.)

10 Coadministration of oncolytic viruses with camptothecins is disclosed in WO 2005/113018 (Wellstat Biologics Corp.). The treatment of cancers using certain mutant herpes viruses in combination with any of numerous anticancer agents, including irinotecan and topotecan, is disclosed in U.S. Patent Publication No. 2002/0071832 (Fong, et al.), paragraphs 7 and 40. Methods of treating neoplasias using target cell-specific adenoviral vectors in combination with antineoplastic agents, including
15 irinotecan or topotecan, are disclosed in U.S. Patent Publication No. 2003/0068307 (Yu, et al.) page 13. See also Nemunaitis, et al., *Cancer Gene Ther.* (2003) 10(5): 341-352; and Meck, et al., *Cancer Res.* (2001) 61(13): 5083-5089.

20 Coadministration of irinotecan, 5-fluorouracil (5-FU) and leucovorin (LV) is disclosed in: Teufel et al., 2004 (*BMC Cancer* 4:38); Tournigand et al., 2004 (*J Clin Oncol* 2:229-237); Andre et al., 1999 (*Eur J Cancer* 35:1343-7); Colucci et al., 2005 (*J Clin Oncol* 22); Bouche et al., 2004 (*J Clin Oncol* 22:4319-4328); Ducreux et al., 1999 (*J Clin Oncol* 17:2901-8); Kohne et al., 2005 (*J Clin Oncol* 23); Saltz et al., 1996 (*J Clin Oncol* 14:2959-67); Goto et al., 2004 (*Int J Clin Oncol* 9:354-8). Additional
25 combinations of irinotecan and 5-FU are listed in Table 3 of Vanhoefer et al., 2001 (*J Clin Oncol* 19:1501-18) and in Sastre et al., 2005 (*Cancer Chemother Pharmacol* 55:453-60).

30 Any discussion of documents, acts, materials, devices, articles or the like which has been included in the present specification is not to be taken as an admission that any or all of these matters form part of the prior art base or were common general knowledge in the field relevant to the present disclosure as it existed before the priority date of each claim of this application.

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SUMMARY OF THE INVENTION

In a first aspect, the invention provides a method of treating a mammalian subject having a neoplasm, comprising administering to the subject a replication competent
5 Newcastle Disease Virus, a fluoropyrimidine, and a camptothecin compound in one or more cycles in a combined amount effective to treat the subject.

This invention provides a method for treating a mammalian subject having a neoplasm, comprising administering to the subject a virus, a fluoropyrimidine compound and a
10 camptothecin compound in a combined amount effective to treat the subject; wherein the virus is selected from the group consisting of a Newcastle disease virus, a measles virus, a vesicular stomatitis virus, an influenza virus, a Sindbis virus, a picornavirus, and a myxoma virus.

15 This invention provides for the use of a virus and/or a fluoropyrimidine and/or a camptothecin compound in the manufacture of a medicament for treating, in combination with the other ingredients mentioned, a subject having a neoplasm; wherein the virus is selected from the group consisting of a Newcastle disease virus, a measles virus, a vesicular stomatitis virus, an influenza virus, a Sindbis virus, a
20 picornavirus, and a myxoma virus. The medicament can contain one, two or all three of the ingredients listed.

BRIEF DESCRIPTION OF THE FIGURES

25 Figure 1. Graph of average tumor volumes of SW620 colon carcinoma xenografts as a function of time after treatment initiation comparing the activity of the triple combination of NDV ($1E+09$ PFU) + Irinotecan (25 mg/kg) + 5-FU (25 mg/kg) versus either agent alone or to vehicle alone.

30 Figure 2. Graph of average tumor volumes of SW620 colon carcinoma xenografts as a function of time after treatment initiation comparing the activity of the triple combination of NDV ($1E+09$ PFU) + Irinotecan (25 mg/kg) + 5-FU (25 mg/kg) versus each doublet (NDV + Irinotecan; Irinotecan + 5-FU; NDV + 5-FU) or to vehicle alone.

35 Figure 3. Graph of average tumor volumes of SW620 colon carcinoma xenografts as a function of time after treatment initiation comparing the activity of the triple

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combination of NDV (1E+09 PFU) + Irinotecan (15 mg/kg) + 5-FU (100 mg/kg)
versus either agent alone or to vehicle alone.

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Figure 4. Graph of average tumor volumes of SW620 colon carcinoma xenografts as a function of time after treatment initiation comparing the activity of the triple combination of NDV (1E+09 PFU) + Irinotecan (15 mg/kg) + 5-FU (100 mg/kg) versus each doublet (NDV + Irinotecan; Irinotecan + 5-FU; NDV + 5-FU) or to vehicle alone.

DETAILED DESCRIPTION OF THE INVENTION

As used herein the transitional term "comprising" is open-ended. A claim utilizing this term can contain elements in addition to those recited in such claim. Thus, for example, the claims can read on treatment regimens that also include other therapeutic agents or therapeutic virus doses not specifically recited therein, as long as the recited elements or their equivalent are present. Thus, as used herein, throughout this specification the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

As used herein "NDV" is an abbreviation for Newcastle Disease Virus. As used herein "DLT" is an abbreviation for dose limiting toxicity. As used herein the term "plaque-forming unit" (PFU) means one infectious virus particle. As used herein "BPFU" means billion PFUs. As used herein "PP" means plaque-purified. Thus, for example PPMK107 means plaque-purified Newcastle Disease virus strain MK107. As used herein "PFU/m²", which is a standard unit for expressing dosages, means PFUs per square meter of patient surface area. As used herein the term "replication-competent" virus refers to a virus that produces infectious progeny in cancer cells.

In an embodiment of this invention the virus is replication-competent.

In accordance with this invention, when the virus is a Newcastle Disease Virus it can be of low (lentogenic), moderate (mesogenic) or high (velogenic) virulence. The level of virulence is determined in accordance with the Mean Death Time in Eggs (MDT) test. (Alexander, "Chapter 27: Newcastle Disease" in Laboratory Manual for the Isolation and Identification of Avian Pathogens, 3rd ed., Purchase, et al. eds. (Kendall/Hunt, Iowa), page 117.) Viruses are classified by the MDT test as lentogenic

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(MDT>90 hours); mesogenic (MDT from 60-90 hours); and velogenic (MDT<60 hours). Mesogenic NDV is currently preferred.

In accordance with this invention, any conventional route or technique for administering viruses to a subject can be utilized. For examples of routes of administration refer to WO 00/62735. In one embodiment of this invention, the virus is administered systemically, for example intravenously. For intravenous administration of a therapeutic virus in accordance with this invention, preferably the virus is a mesogenic strain of Newcastle Disease Virus. In a preferred embodiment of this invention, from 12×10^9 to 120×10^9 PFU/m² per dose of a mesogenic strain of Newcastle Disease virus is administered intravenously to a human subject, more preferably from 12×10^9 to 48×10^9 PFU/m² per dose. As used herein "mg/m²" means milligrams per square meter of patient surface area.

In embodiments of this invention the picornavirus is a poliovirus, an echovirus, or a coxsackievirus. Examples of coxsackieviruses that are suitable in accordance with this invention include the following types: A21, A13, A15 and A18. Examples of suitable echoviruses include echovirus type 1.

The use of fluoropyrimidines as anticancer agents is reviewed in Petty & Cassidy (2004) *Curr. Cancer Drug Targets*, 4:191-204; and Lamont and Schilsky (1999) *Clin. Cancer Res.* 5:2289-2296. These agents are fluorinated pyrimidines which have antitumor activity through several mechanisms including inhibition of RNA synthesis and function, inhibition of thymidylate synthase activity and incorporation into DNA.

As used herein the terms "fluoropyrimidine" or "fluoropyrimidine compound" mean one or more of the following: 5-fluorouracil (5-FU); capecitabine; 5-fluoro-2'-deoxyuridine (FudR); ftorafur; emitefur; eniluracil/5-FU; S-1 (a combination of the 5-FU prodrug ftorafur and two 5-FU modulators called 5-chloro-2,4-dihydroxypuridine and oxonic acid in a molar ratio of 1:0.4:1); and UFT (a combination of ftorafur, and uracil in a 1:4 molar ratio) (Lamont and Schilsky, 1999). 5-FU is often given with the drug leucovorin to enhance the cytotoxic effects of 5-FU (see, for example, Jolivet, 1995, *Eur J Cancer* 31A:1311-1315 and Rustum et al., 1998; *Cancer J Sci Am* 4:12-18). The dosing and administration techniques and schedules for fluuoropyrimidines and for Leucovorin are well known in the art (See, e.g. Vincent et al., 1999 (*Anticancer Drugs* 10:337-54); Jolivet, 1995), and their optimization for a specific

patient is within the ability of the skilled clinician. Bolus dosing with 5-FU is usually administered to human patients in a dosage amount of from 370 to 500 mg/m² daily for 5 days every 4 to 5 weeks, or more preferably 500 mg/m² weekly. In an embodiment of this invention one or more doses of 5-fluorouracil are given by

5 continuous infusion over a period of at least 22 hours per dose. Continuous dosing with 5-FU includes an intravenous bolus dose of 400 mg/m² followed by 600 mg/m² over 22 hours. In another embodiment, a bolus dose of 400 mg/m² is followed by a dose of 2400 mg/m² administered over 46 hours. Leucovorin is usually administered to human patients in a dosage amount of from 200 to 500 mg/m² administered

10 immediately prior to or during the 5-FU doses.

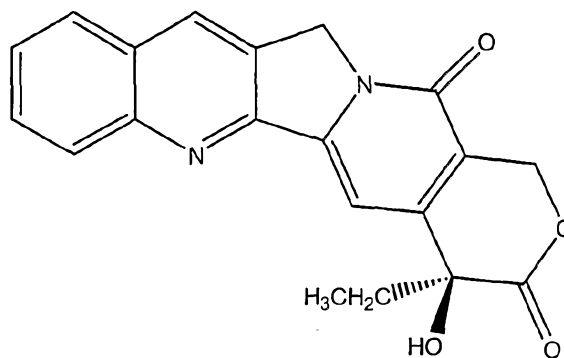
The use of camptothecins as anticancer agents is reviewed in Garcia-Carbonero, et al., Clin. Cancer Res. (March 2002) 8: 641-661; and in Pizzolato JF and Saltz LB, The camptothecins. Lancet 2003 361:2235-42. Camptothecins have antitumor activity

15 based on their binding to and inhibition of topoisomerase I, a nuclear enzyme which reduces torsional stress during DNA replication and which has an important role in DNA replication. Topotecan and irinotecan have been approved for clinical use by the U.S. Food and Drug Administration (FDA). Other camptothecins are in development as cancer therapeutics (Ulukan and Swaan, (Camptothecins: a review of

20 their chemotherapeutic potential. Drugs, 2002, 62:2039-57); and Garcia-Carbonero and Supko, 2002).

As used herein the term "camptothecin compound" means that class of compounds considered to be camptothecins, camptothecin analogs, camptothecin derivatives or

25 camptothecin conjugates. These compounds are based on the characteristic five-ring backbone of camptothecin:



In accordance with this invention any camptothecin compound can be utilized. Examples of camptothecin compounds include irinotecan (CAMPTOSAR; 7-ethyl-
5 10-[4-(1-piperidino)-1-piperidino]-carbonyloxycamptothecin), topotecan (HYCAMPTIN; (S)-9-*N,N*-dimethylaminoethyl-10-hydroxycamptothecin), 9-aminocamptothecin (9-amino-20(*S*)-camptothecin), 9-nitrocamptothecin (also called rubitecan), lurtotecan (7-(4-methylpiperazinomethylene)-10,11-ethylenedioxy-20(*S*)-camptothecin), exatecan, karenitecin, and a homocamptothecin. The structures and
10 clinical information for some camptothecin compounds can be found in Garcia-Carbonero, et al., Clin. Cancer Res. (March 2002) 8: 641-661. Examples of camptothecin compounds can also be found in U.S. Patent No. 4,604,463, No. 6,403,569, and No. 5,004,758, and in WO 2004/012661, WO 2003/101998, WO 2003/101996, WO 2003/101406, WO 2003/093274, WO 2003/086471, WO
15 01/76597, WO 01/64194, WO 00/70275, WO 00/53607, WO 99/17805, WO 99/17804, WO 99/05103, WO 98/35969, WO 97/28164, WO 97/25332, WO 97/16454, the contents of all of which are incorporated herein by reference.

In accordance with the combination therapy of this invention the camptothecin
20 compound can be administered from one month before administration of the virus until one month after administration of the virus. In more specific embodiments the camptothecin compound and the virus are administered to the subject within a single twenty-four hour period; or the camptothecin compound is administered from twenty-four hours to one month, preferably from twenty-four hours to one week, before
25 administration of the virus; or the camptothecin compound is administered to the subject from twenty-four hours to one month, preferably from twenty-four hours to one week, after administration of the virus.

“Chemotherapy is generally given at regular intervals called cycles. A cycle may involve one dose followed by several days or weeks without treatment. This allows normal cells in the body time to recover from the drug’s side effects. Alternatively, 5 doses may be given several days in a row, or every other day for several days, followed by a period of rest. . . . the number of cycles . . . may be determined before treatment starts . . . or may be flexible, in order to take into account how the treatment affects the cancer.” (from “Planning Drug Doses and Schedules”, on website of American Cancer Society, visited May 23, 2005). In an embodiment of this invention 10 the virus, fluoropyrimidine and camptothecin compounds are administered in one or more cycles, more preferably two or more cycles. A single cycle can last, for example, for 8 weeks, 6 weeks, 30 days, or 3 weeks.

Tables 3 through 6 below show representative dosing schedules, which are also 15 suitable in connection with viruses, fluoropyrimidines and camptothecins other than the exemplified ones, for administering amounts that are the same or different as shown in the examples. Accordingly, in further embodiments of this invention, the virus, fluoropyrimidine, and camptothecin compounds are administered in a cycle according to the schedule shown in any one of Tables 3 through 6.

20 The dosing and administration techniques and schedules for camptothecins and anti-cancer viruses are known in the art (See, e.g. Garcia-Carbonero, et al.; WO 00/62735; WO 2004/000209; and Pecora, et al., J. Clin. Oncol. (2002) 20(9): 2251-2266), and their optimization for a specific patient is within the ability of the skilled clinician.

25 Irinotecan is usually administered to human patients in a dosage amount of from 62.5 to 125 mg/m² four times per week, or more preferably 80 to 125 mg/m² four times per week; or from 300 to 350 mg/m² once every three weeks, or more preferably 300 to 350 mg/m² once every three weeks.

30 The subject that is treated in accordance with this invention can be either a human subject or a non-human mammalian subject. In accordance with this invention, any neoplasm can be treated, including but not limited to the following: rectal cancer, pelvic cancer, colon cancer, lung cancer, breast cancer, prostate cancer, glioblastoma, renal cancer, pancreatic cancer, head and neck cancer, endometrial cancer,

neuroblastoma, carcinoid, melanoma, ovarian cancer, sarcoma, cancer of the gastro-esophageal junction, gastric cancer, esophageal cancer, liver cancer, and cervical cancer.

- 5 Although monitoring the treatment is not an essential aspect of the invention, there are techniques for measuring the therapeutic effects of the treatment. These include, measuring the size of the tumor after administration of the virus, and a decrease in tumor size is a positive result.
- 10 The invention will be better understood by reference to the following examples, which illustrate but do not limit the invention described herein. In the following examples the NDV is a triple-plaque purified MK107, which is an attenuated (mesogenic) version of Newcastle Disease Virus, described more fully in International Patent Publication WO 00/62735, published October 26, 2000 (Pro-
- 15 Virus, Inc.). The entire contents of WO 00/62735 and U.S. Provisional Application No. 60/565,631, filed April 27, 2004 are hereby incorporated herein by reference.

EXAMPLES

- 20 EXAMPLE 1. NDV in combination with 5-fluorouracil. (5-FU).
Athymic mice were injected subcutaneously with 10 million human HT1080 fibrosarcoma cells. Seven days later when the tumors were approximately 120 to 150 mm³ in size, animals were randomized and treated with one of 10 different treatment regimens (see Table 1 for details) that included suboptimal doses of intravenous NDV
- 25 (2E+07 or 1E+08 PFU), 100 mg/kg of bolus 5-fluorouracil (5-FU) given intraperitoneally, and subcutaneous implantation of a pellet containing 5 mg of 5-FU (Catalog #Z-190, Innovative Research of America; Sarasota, Florida) or placebo pellets (Catalog #C-111, Innovative Research of America; Sarasota, Florida) and other vehicle controls. If pellets were implanted these were performed at a remote
- 30 site from the subcutaneous tumors. The date of the first treatment is indicated as Day 0 in Table 1 below. 5-FU pellets were used to provide a slower release of 5-FU than bolus dosing. These 5-FU pellets were used to approximate the effects of continuous infusion of 5-FU or administration of an oral analog of 5-FU such as capecitabine over several days.

Table 1. Treatment regimens tested in tumor-bearing athymic mice.

<i>Group</i>	<i>Number of Mice</i>	<i>Treatment, Day 0</i>	<i>Treatment, Day 2</i>	<i>Treatment, Day 4</i>
1	11	M/L ^a	5FU pellet	M/L ^a
2	11	M/L ^a	Pellet control	M/L ^a
3	11	M/L ^a	5FU pellet	NDV, 1E+08
4	11	M/L ^a	Pellet control	NDV, 1E+08
5	11	M/L ^a	5FU bolus	NDV, 1E+08
6	11	M/L ^a	5FU bolus	M/L ^a
7	11	M/L ^a	Bolus control ^b	NDV 1E+08
8	11	M/L ^a	Bolus control ^b	M/L ^a
9	11	NDV, 2E+07	5FU bolus	M/L ^a
10	11	NDV, 2E+07	Bolus control ^b	M/L ^a

^a M/L: Mannitol(5%)/lysine(1%) vehicle control

^b Bolus control: Water for injection

- 5 The incidence of complete tumor regression (CR, 100% tumor reduction was much higher in the group receiving both NDV and 5-FU (whether by bolus dosing or by pellet) than either 5-FU alone or NDV alone see Tables 2a-c.

Table 2a. Treatment of tumor-bearing mice with bolus 5-FU two days after treatment with NDV yields greater tumor responses than either agent alone.

<i>Group</i>	<i>Treatment</i>	Complete Tumor Responses (CR), %
6	Bolus 5-FU	0%
8	Control bolus	0%
9	Both NDV and bolus 5-FU	63%
10	NDV and control bolus	18%

5 Table 2b. Treatment of tumor-bearing mice with bolus 5-FU two days before treatment with NDV yields greater tumor responses than either agent alone.

<i>Group</i>	<i>Treatment</i>	Complete Tumor Responses (CR), %
5	Both NDV and bolus 5-FU	36%
6	Bolus 5-FU	0%
7	NDV and control bolus	0%
8	Control bolus	0%

Table 2c. Treatment of tumor-bearing mice with a 5-FU pellet two days before treatment with NDV yields greater tumor responses than either agent alone.

<i>Group</i>	<i>Treatment</i>	Complete Tumor Responses (CR), %
1	Pellet 5-FU	0%
2	Control Pellet	0%
3	Both NDV and pellet 5-FU	27%
4	NDV and control pellet	0%

5 EXAMPLE 2. NDV in combination with irinotecan, 5-fluorouracil (5-FU) and leucovorin.

Cancer patients are treated with NDV followed by treatment with irinotecan, leucovorin and 5-fluorouracil. In each 6 week cycle, NDV treatment consist of 9 to 12 total intravenous treatments given over 5 weeks followed by a one week rest period (see Table 3 below). The first dose of each cycle consists of 12 to 24 billion PFU/m² (administered over 3 hours for course 1 and over 1 hour for all other courses) followed by additional doses of between 24 to 48 billion PFU/m² (each dose administered over 1 hour). Irinotecan (180 mg/m² over 90 minutes intravenously) is given every other week beginning during week 2 of cycle 1 (As an example, see Table 8 below). Leucovorin (400 mg/m² over 120 minutes intravenously) is given concurrently with each dose of irinotecan and followed immediately afterwards by an intravenous bolus dose of 5-FU (400 mg/m²) and then a continuous intravenous infusion of 5-FU (2400 mg/m²) over 46 hours using an intravenous pump. Additional 6 week courses (also termed cycles) of NDV, irinotecan, 5-FU and leucovorin are given to the patients.

Table 3. Combination of treatment of NDV using irinotecan (80 to 125 mg/m²), leucovorin, and 5-FU. Cycles of treatment are repeated every 6 weeks.

<i>Cycle</i>	<i>Week</i>	<i>NDV?</i>	<i>Irinotecan/Leucovorin/5FU?</i>
1	1	Yes, two doses	No
	2	Yes, two to 3 doses	No
	3	Yes, two to 3 doses	Yes
	4	Yes, one to 2 doses	No
	5	Yes, two to 3 doses	Yes
	6	No	No
2	1	Yes, two doses	Yes
	2	Yes, two to 3 doses	No
	3	Yes, two to 3 doses	Yes
	4	Yes, one to 2 doses	No
	5	Yes, two to 3 doses	Yes
	6	No	No

5 EXAMPLE 3. NDV in combination with irinotecan, 5-fluorouracil (5-FU) and leucovorin.

Colorectal cancer patients are treated as in Example 2 with the NDV, irinotecan, leucovorin and 5-fluorouracil with the schedule as given as in Tables 4-6 below. In each 6 week cycle, NDV treatment consist of 10 total intravenous treatments given over 5 weeks followed by a one week rest period (see Tables 4-6 below). The first dose of each cycle consists of 12 to 24 billion PFU/m² (administered over 3 hours for course 1 and over 1 hour for all other courses) followed by additional doses of between 24 to 48 billion PFU/m² (each dose administered over 1 hour). Irinotecan (180 mg/m² over 90 minutes intravenously) is given every other week beginning during week 2 of cycle 1. Leucovorin (400 mg/m² over 120 minutes intravenously) is given concurrently with each dose of irinotecan and followed immediately afterwards by an intravenous bolus dose of 5-FU (400 mg/m²) and then a continuous intravenous infusion of 5-FU (2400 mg/m²) over 46 hours using an intravenous pump. Additional 6 week courses (also termed cycles) of NDV, irinotecan, 5-FU and leucovorin are given to the patients.

Table 4. Combination of treatment of colorectal cancer patients with NDV, irinotecan (80 to 125 mg/m²), leucovorin, and 5-FU. Cycles of treatment are repeated every 6 weeks.

<i>Cycle</i>	<i>Week</i>	<i>NDV?</i>	<i>Irinotecan/Leucovorin/5FU?</i>
1	1	Yes, two doses given 3 days apart	No
	2	Yes, 3 doses given 2 days apart	No
	3	Yes, two doses given 4 days apart	Yes
	4	Yes, one dose	No
	5	Yes, two doses given 4 days apart	Yes
	6	No	No
2 and higher	1	Yes, two doses given 4 days apart	Yes
	2	Yes, two doses given 4 days apart	No
	3	Yes, two doses given 4 days apart	Yes
	4	Yes, two doses given 4 days apart	No
	5	Yes, two doses given 4 days apart	Yes
	6	No	No

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Table 5. Typical Dosing Schedule - Cycle 1

Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
1 NDV Dose 1	2	3	4 NDV Dose 2	5	6	7
8 NDV Dose 3	9	10 NDV Dose 4	11	12 NDV Dose 5	13	14
15 NDV Dose 6	16	17 Irinotecan/ LV/5FU	18	19 NDV Dose 7	20	21
22 NDV Dose 8	23	24	25	26	27	28
29 NDV Dose 9	30	31 Irinotecan/ LV/5FU	32	33 NDV Dose 10	34	35
36	37	38	39	40	41	42

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Table 6. Typical Dosing Schedule - Cycles 2 and higher:

Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
1 NDV Dose 1	2	3 Irinotecan/ LV/5FU	4	5 NDV Dose 2	6	7
8 NDV Dose 3	9	10	11	12 NDV Dose 4	13	14
15 NDV Dose 5	16	17 Irinotecan/ LV/5FU	18	19 NDV Dose 6	20	21
22 NDV Dose 7	23	24	25	26 NDV Dose 8	27	28
29 NDV Dose 9	30	31 Irinotecan/ LV/5FU	32	33 NDV Dose 10	34	35
36	37	38	39	40	41	42

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EXAMPLE 4.

Athymic mice were injected subcutaneously with 10 million human SW620 colon carcinoma cells (obtained from American Type Culture Collection, Manassas, VA).

5 Five days later (termed day 0 below) when the subcutaneous tumors were approximately 85 mm³ in size, groups of 11 to 12 animals were randomized into 8 treatment groups:

- NDV + Irinotecan
- NDV + 5-FU
- 10 • NDV + Irinotecan + 5-FU
- Irinotecan
- 5-FU
- Irinotecan + 5-FU
- Vehicle only

15

During Day 0 all mice received either NDV (1E+09 PFU) or vehicle by the intravenous route according to the treatment key provide above. Two days later on Day 2, all mice received a first intraperitoneal injection of either irinotecan (25 mg/kg) or vehicle and then 1 hour later received a second intraperitoneal injection of
20 either 5-FU (25 mg/kg) or vehicle according to the treatment key provided above. The vehicle for NDV was an aqueous solution of 5% mannitol/1% lysine. The vehicle for irinotecan was saline. The vehicle for 5-FU was water for injection. Tumor sizes were periodically determined for all mice using calipers.

25 As shown in Figure 1, the combination of NDV + irinotecan + 5-FU was markedly superior in terms of antitumor activity to either agent alone or to control vehicle. As shown in Figure 2, the combination of NDV + irinotecan + 5-FU was superior in terms of antitumor activity to either of the treatment doublets (NDV + Irinotecan; Irinotecan + 5-FU; NDV + 5-FU).

30

EXAMPLE 5.

This experiment was performed as in Example 4, except for the following: (1) treatment was started seven days after subcutaneous inoculation of the SW620 tumor cells; (2) the subcutaneous tumors were approximately 100 mm³ in size, (3) there were 13 mice per treatment group; (4) the dose of irinotecan as 15 mg/kg instead of 25 mg/kg; and (5) the dose of 5-FU was 100 mg/kg instead of 25 mg/kg. As before, animals were randomized into 8 treatment groups:

- NDV
- 10 • NDV + Irinotecan
- NDV + 5-FU
- NDV + Irinotecan + 5-FU
- Irinotecan
- 5-FU
- 15 • Irinotecan + 5-FU
- Vehicle only

As before, during Day 0 all mice received either NDV (1E+09 PFU) or vehicle by the intravenous route according to the treatment key provide above. Two days later on Day 2, all mice received a first intraperitoneal injection of either irinotecan (15 mg/kg) or vehicle and then 1 hour later received a second intraperitoneal injection of either 5-FU (100 mg/kg) or vehicle according to the treatment key provided above. Tumor sizes were periodically determined for all mice using calipers.

As shown in Figure 3, the combination of NDV + irinotecan + 5-FU was again markedly superior in terms of antitumor activity to either agent alone or to control vehicle. As shown in Figure 4, the combination of NDV + irinotecan + 5-FU was again superior in terms of antitumor activity to either of the treatment doublets (NDV + Irinotecan; Irinotecan + 5-FU; NDV + 5-FU). Furthermore, there was evidence for a synergistic antitumor effect from using all three agents in causing durable tumor regressions. As shown in Table 7, there were more complete tumor regressions (CRs) that were durable (i.e., extending through Day 90) using the triple combination NDV + irinotecan + 5-FU than in any other treatment group including any of the treatment

doublets (NDV + Irinotecan; Irinotecan + 5-FU; NDV + 5-FU) and each of the single agents alone.

- 5 Table 7. Treatment of tumor-bearing mice with the triple combination of NDV + Irinotecan + 5-FU yielded greater durable complete tumor responses than any agent alone or any treatment doublet.

<i>Treatment</i>	Number of Mice	Durable CRs*, %
NDV	13	0%
NDV + Irinotecan	13	23%
NDV + 5-FU	13	0%
NDV + Irinotecan + 5-FU	13	46%
Irinotecan	13	0%
5-FU	13	0%
Irinotecan + 5-FU	13	0%
Vehicle Only	13	0%

* CRs that extended through Day 90.

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CLAIMS:

1. A method of treating a mammalian subject having a neoplasm, comprising administering to the subject a replication competent Newcastle Disease Virus, a fluoropyrimidine, and a camptothecin compound in one or more cycles in a combined amount effective to treat the subject.
2. The method of claim 1, wherein the virus is a mesogenic strain of Newcastle Disease Virus.
3. The method of any preceding claim, wherein the virus is administered intravenously.
4. The method of any preceding claim, wherein the fluoropyrimidine compound is 5-fluorouracil.
5. The method of any preceding claim, further comprising administering to the subject leucovorin.
6. The method of any preceding claim, wherein the 5-fluorouracil is administered by continuous infusion over a period of at least 22 hours per dose.
7. The method of any preceding claim, wherein the camptothecin compound is selected from the group consisting of irinotecan, topotecan, 9-aminocamptothecin, exatecan, karenitecin, rubitecan, lurtotecan, and a homocamptothecin.
8. The method of claim 7, wherein the camptothecin compound is irinotecan.
9. The method of any preceding claim, wherein the virus is a mesogenic strain of Newcastle Disease Virus, the fluoropyrimidine is 5-fluorouracil, and the camptothecin compound is irinotecan.
10. The method of any preceding claim, wherein the virus, the fluoropyrimidine, and the camptothecin compound are administered in two or more cycles.

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- 11. The method of any preceding claim, wherein the camptothecin compound is administered to the subject from twenty-four hours to one month before administration of the virus.
- 5 12. The method of claim 11, wherein camptothecin compound is administered to the subject from twenty-four hours to one week before administration of the virus.
- 13. The method of any preceding claim, wherein the camptothecin compound is administered to the subject from twenty-four hours to one month after administration of
10 the virus.
- 14. The method of claim 13, wherein camptothecin compound is administered to the subject from twenty-four hours to one week after administration of the virus.
- 15 15. The method of any one of claims 1-14, substantially as described herein.

FIGURE 1

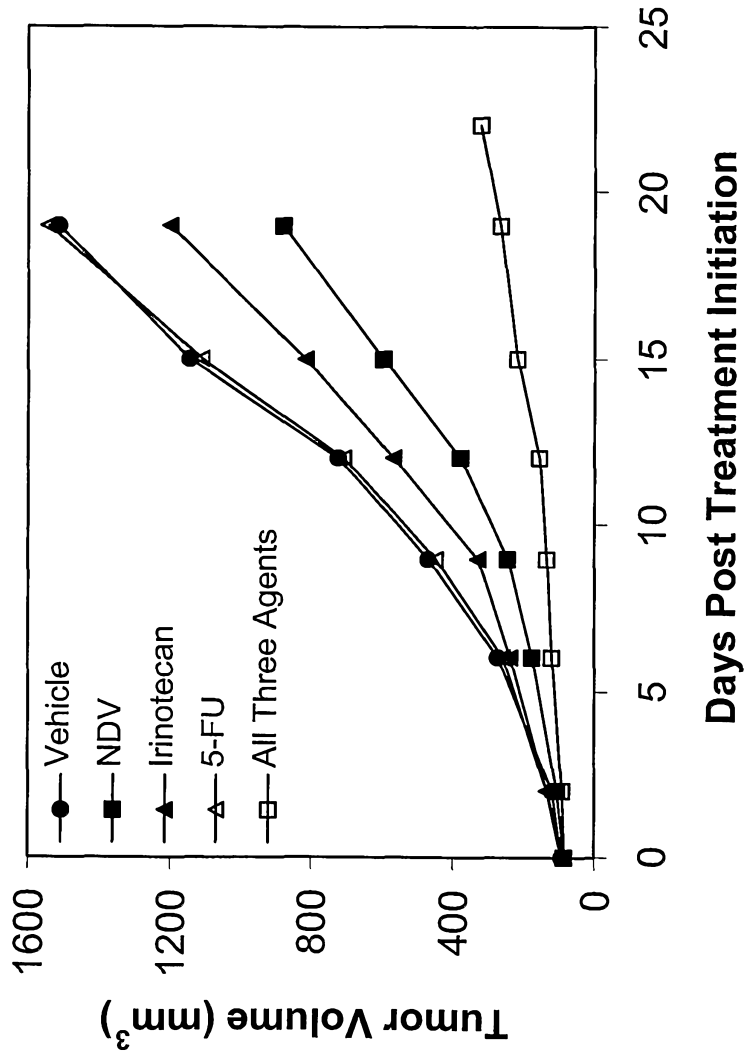


FIGURE 2

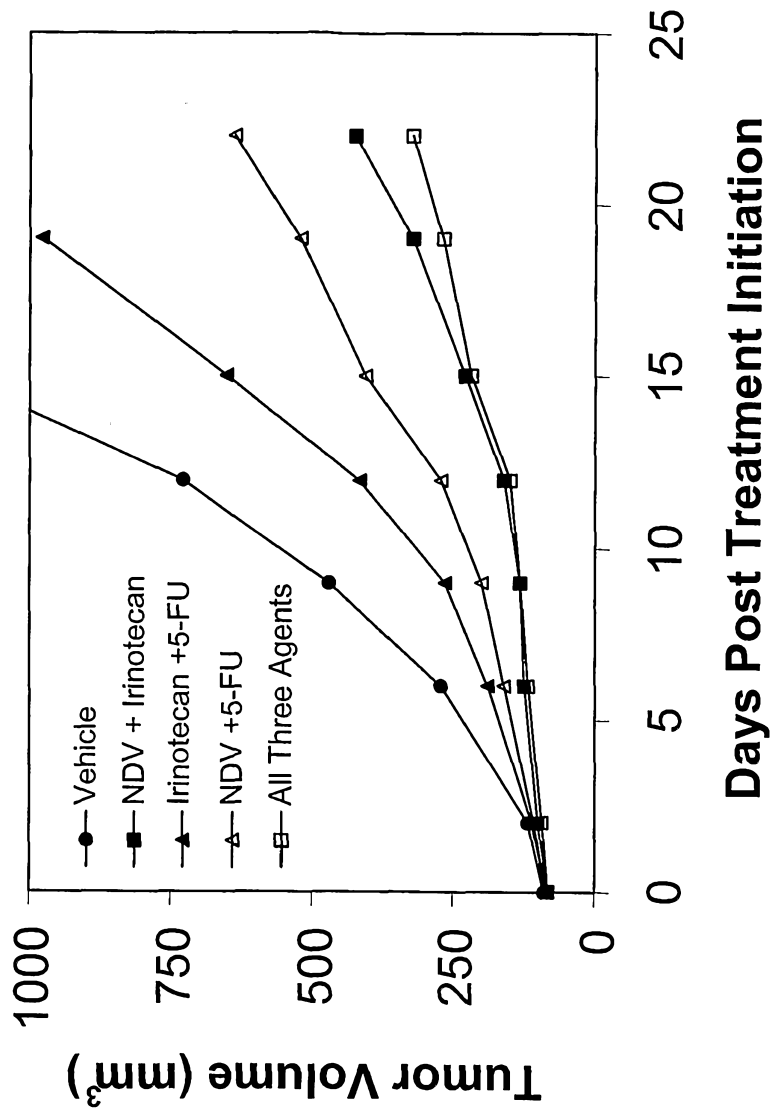


FIGURE 3

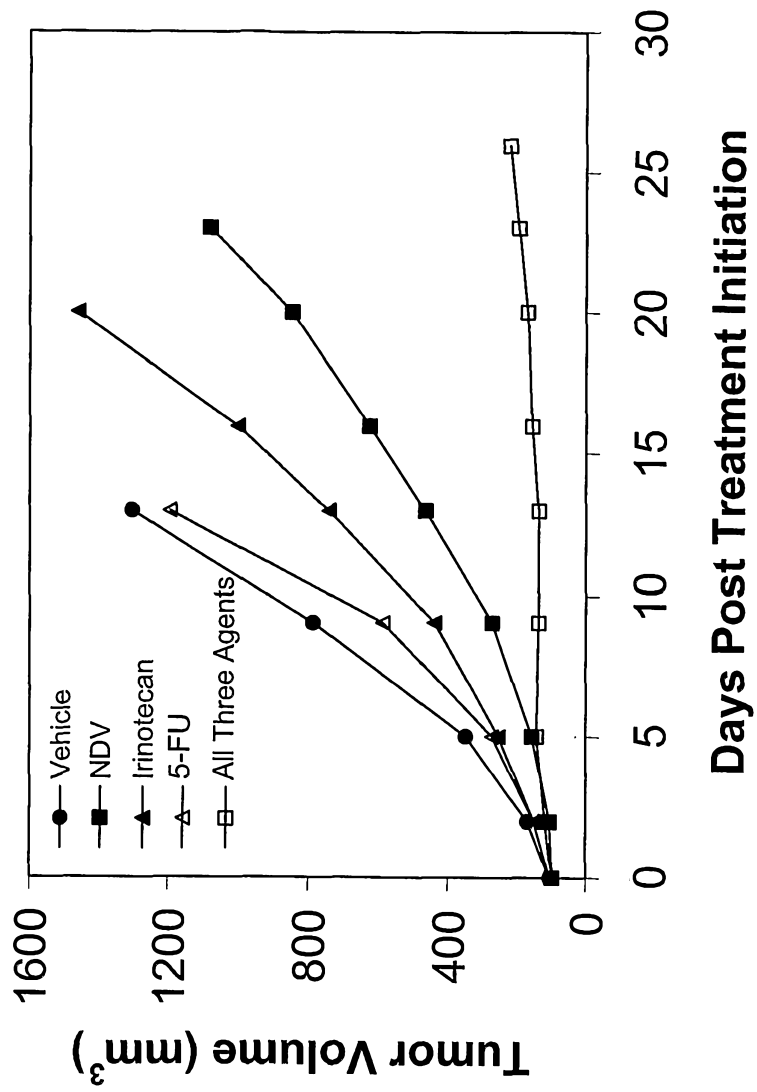


FIGURE 4

