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PRECLINICAL EVALUATION OF BOTH EARLY STAGE AND LATE STAGE ANTITUMOR ACTIVITY IN A SINGLE ANIMAL MODEL

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Abstract:

Cancer model was developed using syngenic model of cancer studies in which three different groups of 6 animals each was made. CT26 cell lines are use for induction of tumor which was purchased from ATCC .One group contains vehicle control group and other two groups was assigned as treatment groups in which one is treated with Avastin 2mg/kg alternate day i.v. treatment for two weeks(early stage) and again for same schedule in late stage similarly other group is treated with Paclitaxel 5mg/kg i.v on alternate day treatment for 5 times (early stage) and same pattern was used for late stage schedule .The result of present study successfully provides a single model to screen anticancer compound in early stage as well as in late stage.

Keywords: Preclinical cancer moder, In vivo cancer model, Early stage cancer model,Late stage cancer model .

Introduction

Colon cancer is the third most common cancer in both men and women, and it is the second leading cause of cancer death in Western countries[1].Colon cancers are adenocarcinomatous tumors that arise from the glands lining the colon's inner wall. Colon cancer is also referred to as colorectal cancer or large bowel cancer. It is responsible for the death of over 55,000 patients annually[2]. Each year, approximately around 102,900 **new cases** of colon cancer are reported[3]. The incidence in both developing and developed countries has been increasing over the past few decades. Mostly it affects the colon, but sometimes it may also affect the rectum, the end portion of the colon. Concurrent chemo-radiation therapy (CCRT) plays an important role in controlling CRC and palliating symptoms.

This is particularly true when aiming for anal preservation in locally advanced disease, and it therefore offers an attractive alternative to surgery, the current mainstay of treatment[4] Consequently, colon cancer remains a major public health priority. The main cause for high mortality rate is that the prognosis for progressed metastatic colon cancer is most unfavorable[5]The main prognostic factor for survival or relapse is tumor staging[6].

5-Fluorouracil was the first drug to show a survival advantage over surgery alone in adjuvant colon cancer[6-12]Bevacizumab (Avastin) is a humanized monoclonal antibody (MoAb) targeting vascular endothelial growth factor. Adding bevacizumab to standard chemotherapy (5-FU/irinotecan, 5-FU/oxaliplatin, 5-FU alone) improves outcomes in patients with mCRC[13-15]Specific bevacizumab-related side effects have been observed: bleeding, hypertension, gastrointestinal (GI) perforation, and arterial thromboembolic events. The addition of bevacizumab has significantly improved the PFS of chemotherapy alone. The magnitude of benefit is higher with irinotecan than with oxaliplatin, and this might be due either to a better synergy or to a more prolonged administration of bevacizumab in the irinotecan trial. Of note, the benefit of bevacizumab appears more pronounced in first-line than in second-line and is not observed in third-line therapy[16,17].

The efficacy of cancer chemotherapy is considerably limited by toxic side effects of anticancer drugs. This limitation results from the fact that conventional chemotherapy exposes both normal and neoplastic cells to identical doses of cytotoxic agents and relies upon the enhanced sensitivity of rapidly dividing cancer cells to achieve preferential killing[18]. Angiogenesis plays a role in early-stage colorectal tumor progression[19] and is required for cancer growth beyond 1–2 mm3[20,21]. Tumor angiogenesis is driven primarily by VEGF:VEGFR2 interaction [22,23]. Vascular endothelial growth factor A (VEGF) is the best characterized member of the VEGF family of growth factors. VEGF is a potent angiogenic factor expressed during development and in tumors [24,25]. The effects of VEGF are mediated by binding to one of its two receptors VEGF receptor 1 or 2 (VEGFR1, VEGFR2) The effect of VEGFR1 activation is less understood, but is thought to be involved in macrophage chemotaxis [26]. Reliance on

the conventional end-point of tumour volume is problematic, since antiangiogenic drugs are cytostatic in action and do not directly induce tumour shrinkage[19,20].

Materials and methods

Media

Cell culturing medium composed of DMEM with L-Glutamine,15mM HEPES buffer, Sodium bi-carbonate & trace elements(purchased from HIMEDIA Laboratories Pvt Ltd.), 10% FBS (Purchased from Biosera) and 1% antibiotic solution [(penicillin, streptomycin solution, purchased from Hyclone Lab Inc.)].

Cell culturing medium for CT26 murine colon carcinoma cells was composed of DMEM, 10% FBS, 1% antibiotic solution (penicillin, streptomycin solution) and filtered glucose.

Flasks

- Small-size flasks (25 cm^2) were filled with 8-10 ml of medium
- Medium-size flasks (75 cm^2) were filled with 20 ml of medium
- Large-size flasks (150 cm^2) were filled with 40 ml of medium

Cell Passage

Cell passaging or splitting is a technique that enables an individual to keep cells alive and growing under cultured conditions for extended periods of time. Cells should be passed when they are 90%-100% confluent. In order for cells to grow beyond confluency, they must be passaged. Upon reaching confluency, cells were washed with PBS and treated with a 0.05%-EDTA tyrpsin solution (BioConcept). Detachment of cells was done using trypsin solution. The cell suspension was transferred into a 15-ml falcon tube and 10 ml medium added. Cells were pelleted by centrifugation (5 min, 1400 rpm), resuspended in fresh medium and divided into culture flasks with medium. Typically, cells were passaged every other day from one flask to a bigger-size flask, or from a large one to three large flasks.

Preparation of Cryotubes

Typically, cryotubes were prepared from one large culture flask of confluent cells as follows:

The medium was removed and the flask washed with 20 ml PBS. Trypsin, 2.5ml was then added and the flask was shaken gently to rinse all cells. The trypsinized cells were transferred into a 15 ml falcon tube and 10 ml medium added to block the reaction. After centrifugation (5 min., 1400 rpm), the supernatant was aspirated and new fresh medium added. After a second wash with PBS, cells were resuspended in 5 ml of cryotube-medium and aliquoted into 10 cryotubes. The medium used for the preparation of cryotubes was composed of DMEM medium, 20% FBS, 10% DMSO (alternatively, a 10% DMSO solution in FBS can be used for cryotube preparation). These tubes were frozen immediately at -80 ºC for at least three days, and then transferred into a liquid nitrogen tank.

Preparation of cells for subcutaneous injection in mice

Medium was aspirated from a flask containing confluent cells. Washing was done using PBS followed by trysinization. The trypsinized cells were transferred into a 15 ml falcon tube and 10 ml medium added. After centrifugation (5 min., 1400 rpm), cells were resuspended in sterile PBS (12 ml) and centrifuged again. The cell pellet was then resuspended in 500-600 µl of PBS and kept in ice, providing material for immediate s.c. injection into 3-4 mice. Cell concentration before injection was determined using a Neubauer-cytometer (Sigma). Approximately 106-107 cells were injected subcutaneously into 6-14 week-old female Balb/c mice.

Induction of tumor

1. Inoculation area of the mice was cleaned and sterilized with ethanol.
2. 1-cc syringe and a 27-gauge needle were used for injecting the cell suspensiom.
3. Cells were mixed and drawn into a syringe without a needle as using a needle causes a strong, negative pressure, which can cause cell damage and lysis.
4. (2.0×10^6) in 200µL cells were injected subcutaneously (s.c.) into the lower flank of the mice.
5. Tumor diameters were measured with digital calipers, and the tumor volume in mm^3 was calculated the following formula:-

   Volume = $(\text{width})^2 \times \text{length}/2$
6. Five out of eight mice showed a good developed tumor

7. The tumor was allowed to grow till maximum size. It was then separated and chopped into fragments.s

Implantation of tumor fragments

1. The chopped fragments were implanted using trochar (instrument used to inject fragments) subcutaneously to the left flank of Balb/c Mice.
2. All procedure of chopping and injecting tumor were done under aseptic condition.
3. Again, the mice were allowed for tumor development.
4. The tumor was visible after one week, when it attained a size of 200-400 mm^3
5. Animals were randomized into three different groups.

Results

Clinical sign of toxicity & Mortality

Animals of all the groups did not show any clinical toxicity signs during cage side observation.

At the starting of Interstage period vehicle control group shows a mortality and at the end of Interstage period same group again shows a mortality . Interestingly Avastin 2mg/kg i.v. treatment group did not show any mortality. Whereas Paclitaxel 5mg/kg group shows single mortality within early stage dosing period

Table 1:- Mortality Chart.

S.no	Group	No. of Animals in group (n)	Survival Data Throughout Early Stage Dosing Period			Survival Data Throughout Inter stage Period		Survival Data Throughout Late Stage Dosing Period	
			Day 01	Day 07	Day 14	Day 21	Day 28	Day 35	Day 42
G1	Vehicle Control	06	6/6	6/6	6/6	5/6	4/6	4/6	4/6
G2	Avastin 2mg/kg i.v.	06	6/6	6/6	6/6	6/6	6/6	6/6	6/6

G3	**Paclitaxel 5mg/kg i.v.**	06	6/6	5/6	5/6	5/6	5/6	5/6	5/6

	Days	**Vehicle Control (G1)**		**Avastin, 2mg/kg (G2)**		**Paclitaxel, 5mg/kg (G3)**	
		Mean Body weight (g)	SD	Mean Body weight (g)	SD	Mean Body weight (g)	SD
Early Stage Dosing	1	25.78	1.14	23.72	1.20	23.75	2.44
	2	26.98	1.19	24.42	1.66	23.67	2.09
	3	26.43	1.29	24.55	1.51	22.47	2.02
	4	26.37	1.43	24.88	1.46	23.85	2.33
	5	26.82	1.46	25.15	1.35	24.73	3.02
	6	26.80	1.26	25.38	1.19	24.98	2.40
	7	26.63	1.14	25.48	1.12	24.63	3.05
	8	26.85	1.18	25.95	0.94	24.82	3.16
	9	27.12	1.18	26.17	1.06	24.93	3.45
	10	28.22	1.28	25.88	1.72	24.60	3.25
	11	27.73	1.61	26.05	1.90	24.93	3.30
	12	27.87	1.70	26.68	1.48	25.45	3.07
	13	27.85	1.69	26.72	1.45	25.45	2.91
	14	27.88	1.57	26.85	1.55	25.48	2.93
Inter Stage Period	15	27.87	1.53	26.93	1.66	25.00	2.09
	16	27.87	1.31	26.88	1.58	25.03	2.09
	17	27.98	1.32	26.90	1.73	25.15	2.04
	18	28.12	1.23	26.95	1.63	25.33	2.04
	19	28.23	1.30	27.02	1.63	25.72	2.42
	20	28.38	1.23	27.12	1.76	26.00	2.38

	21	28.57	1.27	27.30	1.75	26.15	2.26
	22	28.65	1.39	27.28	1.84	26.32	2.23
	23	28.75	1.34	27.37	1.77	26.23	2.12
	24	28.80	1.05	27.38	1.76	26.23	2.25
	25	28.98	1.11	27.47	1.87	26.40	2.26
	26	29.05	1.01	27.58	1.87	26.57	2.25
	27	29.10	1.04	27.60	1.83	26.65	2.31
	28	29.23	0.96	27.65	1.79	26.78	2.32
Late Stge Dosing	29	29.27	1.00	27.80	1.89	26.78	2.36
	30	29.38	0.95	27.80	1.83	26.95	2.27
	31	29.57	0.95	27.72	1.98	27.02	2.26
	32	29.73	1.03	27.55	1.84	27.07	2.35
	33	29.85	1.00	27.48	1.71	27.15	2.30
	34	29.88	0.82	27.37	1.58	27.15	2.20
	35	29.87	0.84	27.23	1.61	27.10	2.18
	36	30.03	0.83	27.15	1.58	27.15	2.14
	37	30.20	0.80	27.05	1.51	27.15	2.20
	38	30.20	0.70	26.90	1.48	27.10	2.28
	39	30.40	0.72	26.70	1.42	27.18	2.52
	40	30.52	0.72	26.72	1.45	27.18	2.55
	41	30.67	0.57	26.60	1.40	27.18	2.38
	42	30.83	0.56	26.55	1.36	27.20	2.38

Mortality data suggest that, there is no compound related toxicity throughout dosing period.

Table 2: Mean Body Weight

5.1 Body Weight

Mean body weight and % change in body weight for each group is represented in Table 2 & 3 respectively. The graphical representation of the same found in Figure 1 & 2.

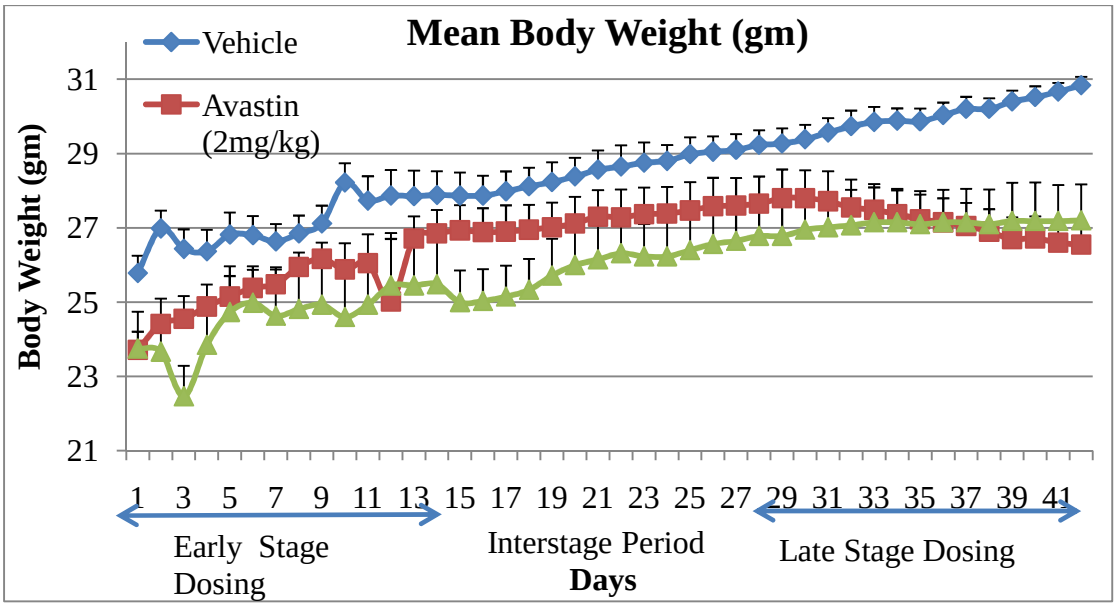

Fig1: Mean Body weight

Mean / MEDIAN tumor volume (TV)

Mean/Median tumor volume data is represented in Table 4 &5 and fig 3&4

On 1st day of treatment mean and median tumor volume of each group was found to be in the series of 103-166 mm^3 and 60-95 mm^3 respectively. The tumor volume of vehicle control group found to be persistently increasing throughout the experimental period conversely each of drug treatment shows tumors growth inhibition when compared to vehicle control group.

		Vehicle Control		**Avastin, 2mg/kg**		**Paclitaxel, 5mg/kg**	
		(G1)		**(G2)**		**(G3)**	
	Days	Mean Tumor Volume (mm3)	SD	Mean Tumor Volume (mm3)	SD	Mean Tumor Volume (mm3)	SD

Early Stge Dosing	1	103.69	39.0	127.24	159.9	165.88	183.8
	3	211.74	119.8	323.38	541.1	198.09	233.9
	5	403.59	255.8	445.58	589.1	243.07	316.2
	7	755.68	550.8	533.61	701.4	341.65	585.3
	9	1357.01	1087.6	787.32	1133.2	484.94	931.9
	11	2132.63	1752.4	1259.33	1970.6	625.81	1254.7
	13	3359.59	2896.2	1543.89	2316.1	954.45	549.2
Inter stage period	15	3907.33	3755.9	1953.91	2755.8	1186.08	2353.8
	17	3962.24	3779.7	1988.08	2780.2	1467.91	2819.9
	19	4292.35	4086.3	2031.94	2824.0	1538.35	2984.9
	21	4639.24	4336.3	2107.03	2928.9	1792.22	3251.5
	23	4808.75	4449.8	2218.66	3006.2	1742.83	3086.5
	25	5291.78	4874.2	2345.30	3162.4	1886.62	3363.7
	27	5429.6	4917.6	2456.35	3254.3	2041.57	3576.9
Late Stge Dosing	29	5465.5	4905.8	2571.37	3358.8	1934.94	3233.0
	31	5634.0	4877.4	2670.75	3457.3	1819.72	2957.0
	33	5615.8	4779.1	2633.52	3418.9	1913.60	3184.2
	35	5517.8	4588.2	2406.54	3032.6	1907.67	3195.1
	37	5809.5	4725.1	2424.36	3122.9	1735.74	2870.2
	39	6086.4	4889.3	2469.48	3232.8	1602.27	2638.0
	41	6354.9	5068.7	2271.01	2861.1	1626.62	2701.9

Table: 4. - Mean Tumor Volume (mm3)

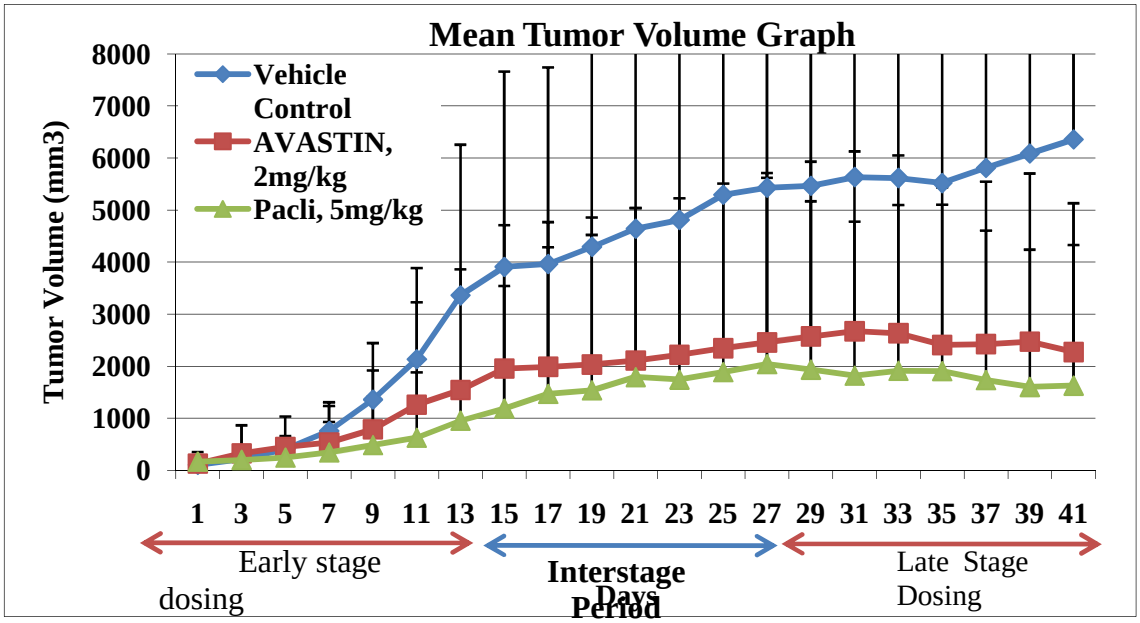

Fig 3. - Mean Tumor Volume

	Days	Vehicle Control (G1)	Avastin, 2mg/kg (G2)	Paclitaxel, 5mg/kg (G3)
Early Stage Dosing	1	95.49	60.42	67.81
	3	235.57	128.39	99.67
	5	448.41	241.69	117.50
	7	847.99	259.59	47.09
	9	1558.12	262.26	118.50
	11	2559.88	348.72	57.77
	13	3648.91	501.36	72.25

Inter stage period	15	4147.59	732.69	186.83
	17	4288.34	760.68	186.83
	19	4640.90	794.19	211.82
	21	5336.93	811.26	242.27
	23	5832.04	863.21	7.56
	25	6323.46	964.49	285.31
	27	6678.0	1078.34	304.90
Late stage Dosing	29	6735.3	1177.53	361.39
	31	7281.1	1189.37	333.37
	33	7650.5	1217.94	313.00
	35	7996.7	1184.45	194.78
	37	8799.3	1152.22	316.24
	39	9128.2	1134.76	236.97
	41	9736.3	1106.49	150.37

Table: 5. - Median Tumor Volume

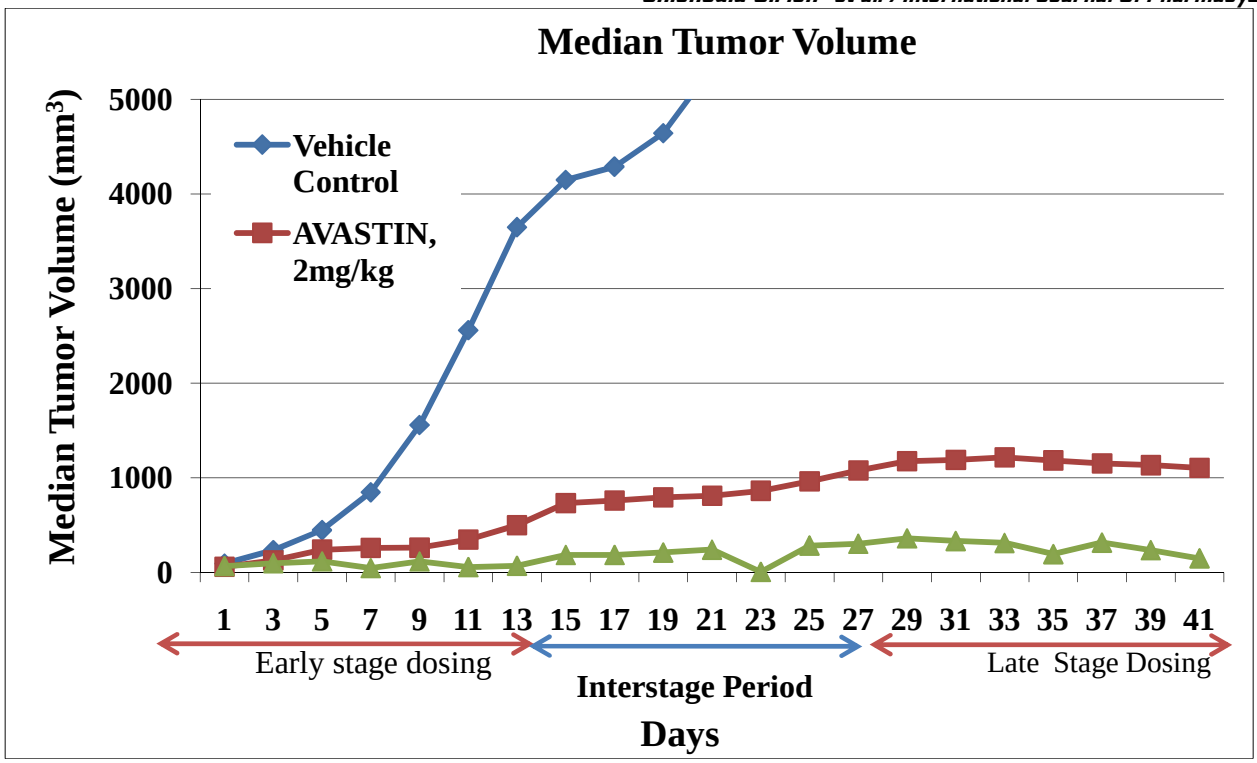

Fig4:Median Tumor Volume

Mean / MEDIAN Tumor Growth Inhibition (TGI)

Mean/Median tumor growth inhibition as compared to vehicle control for all the group was calculated from tumor volume data and represented in table 6 & 7 and fig 5 & 6.

At the end of dosing period in early stage , Avastin 2mg/kg alternate day intravenous treatment shows highest mean tumor growth inhibition of 49.99 %. Paclitaxel 5mg/kg intravenous treatment on alternate day for five times shows maximum mean tumor growth inhibition of 69.6 % .Whereas at the end of dosing period in late stage,Avastin 2mg/kg I.V on alternate day shows maximum mean tumor growth inhibition of 58.2%.Interestingly Paclitaxel 5mg/kg intravenous treatment on alternate day for five times shows maximum mean tumor growth inhibition of 70.1%.The same parameters if we observe at the end of interstage period ,it was found to be 42.6% for Avastin 2mg/kg I.V on alternate days and for Paclitaxel 5mg/kg intravenous treatment on alternate day for five times the tumor growth inhibition was found to be 52.3%

	Days	Vehicle Control (G1)	Avastin, 2mg/kg (G2)	Paclitaxel, 5mg/kg (G3)
Early Stage Dosing	1	0	-22.71	-59.98
	3	0	-52.72	6.45
	5	0	-10.40	39.77
	7	0	29.39	54.79
	9	0	41.98	64.26
	11	0	40.95	70.66
	13	0	54.05	71.59
Inter stage Period	15	0	49.99	69.64
	17	0	49.82	62.95
	19	0	52.66	64.16
	21	0	54.58	61.37
	23	0	53.86	63.76
	25	0	55.68	64.35
	27	0	54.8	62.4
Late Stage Dosing	29	0	53.0	64.6
	31	0	52.6	67.7
	33	0	53.1	65.9
	35	0	56.4	65.4
	37	0	58.3	70.1
	39	0	59.4	73.7
	41	0	64.3	74.4

Table: 6. - Mean Tumor Growth Inhibition

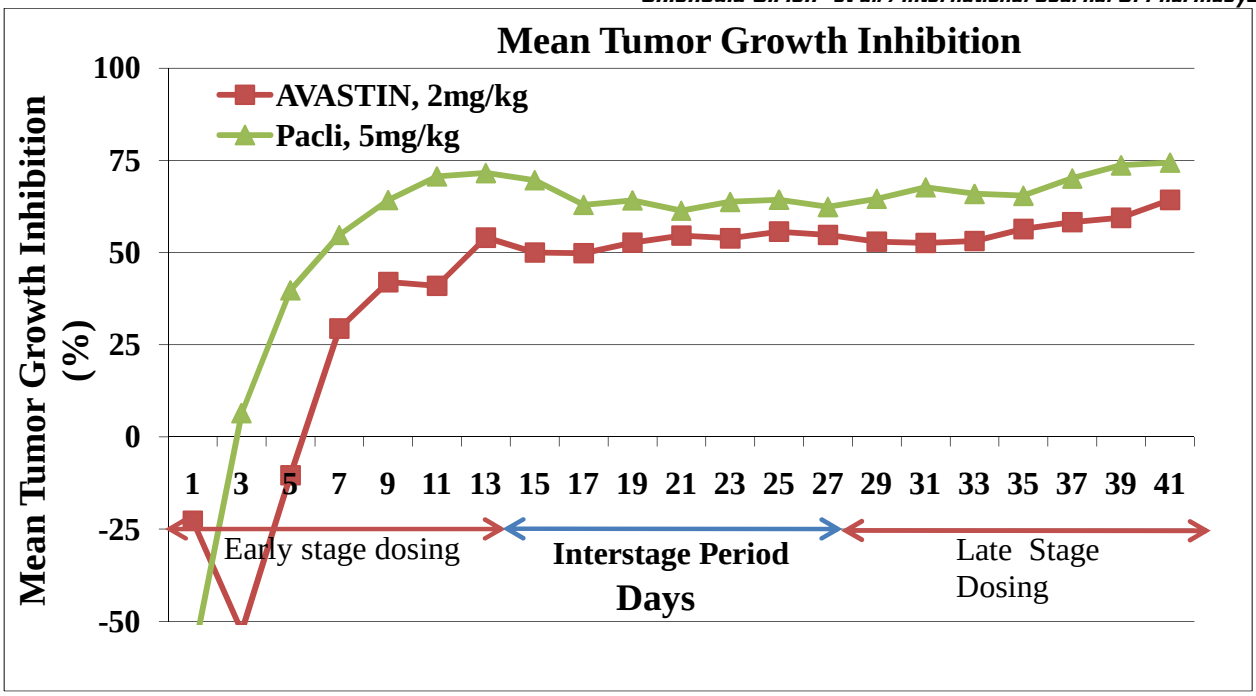

Fig 5: Mean Tumor Growth Inhibition

	Days	Vehicle Control (G1)	Avastin, 2mg/kg (G2)	Paclitaxel, 5mg/kg (G3)
Early Stage Dosing	1	0.0	36.7	29.0
	3	0.0	45.5	57.7
	5	0.0	46.1	73.8
	7	0.0	69.4	94.4
	9	0.0	83.2	92.4
	11	0.0	86.4	97.7
	13	0.0	86.3	98.0
Inter stage period	15	0.0	82.3	95.5
	17	0.0	82.3	95.6
	19	0.0	82.9	95.4
	21	0.0	84.8	95.5

	23	0.0	85.2	99.9
	25	0.0	84.7	95.5
	27	0.0	83.9	95.4
Late Stage Dosing	29	0.0	82.5	94.6
	31	0.0	83.7	95.4
	33	0.0	84.1	95.9
	35	0.0	85.2	97.6
	37	0.0	86.9	96.4
	39	0.0	87.6	97.4
	41	0.0	88.6	98.5

Table: 7. - Median Tumor Growth Inhibition

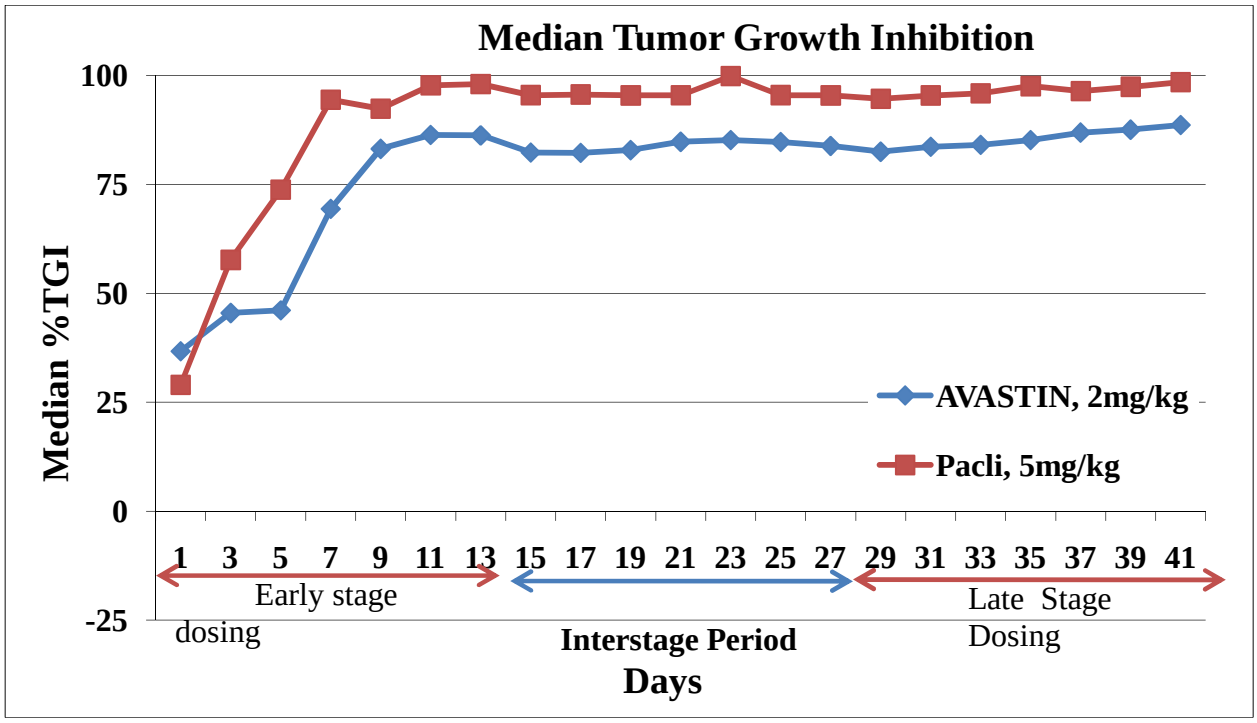

Fig 6:Median Tumor Growth Inhibition

Mean / Median % T/C

Mean and median % T/C for all the groups was calculated from tumor volume data and represented in table 8 & 9 and fig 7 & 8 respectively.

Avastin 2mg/kg alternate day intravenous treatment at the end of early stage shows mean % T?C value of 46.0, while Paclitaxel 5mg/kg intravenous treatment on alternate day for five times treatment, shows a value of 28.4 on last day of early stage .

If we see the same parameters in late stage it was 41.8 for Avastin 2mg/kg and 29.9 for Paclitaxel 5mg/kg.

	Days	Vehicle Control (G1)	Avastin, 2mg/kg (G2)	Paclitaxel, 5mg/kg (G3)
Early Stage Dosing	1	100	122.7	160.0
	3	100	152.7	93.6
	5	100	110.4	60.2
	7	100	70.6	45.2
	9	100	58.0	35.7
	11	100	59.1	29.3
	13	100	46.0	28.4

Inter stage period	15	100	50.0	30.4
	17	100	50.2	37.0
	19	100	47.3	35.8
	21	100	45.4	38.6
	23	100	46.1	36.2
	25	100	44.3	35.7
	27	100	45.2	37.6
Late Stage Dosing	29	100	47.0	35.4
	31	100	47.4	32.3
	33	100	46.9	34.1
	35	100	43.6	34.6
	37	100	41.7	29.9

	39	100	40.6	26.3
	41	100	35.7	25.6

Table: 8. - Mean % T/C Data

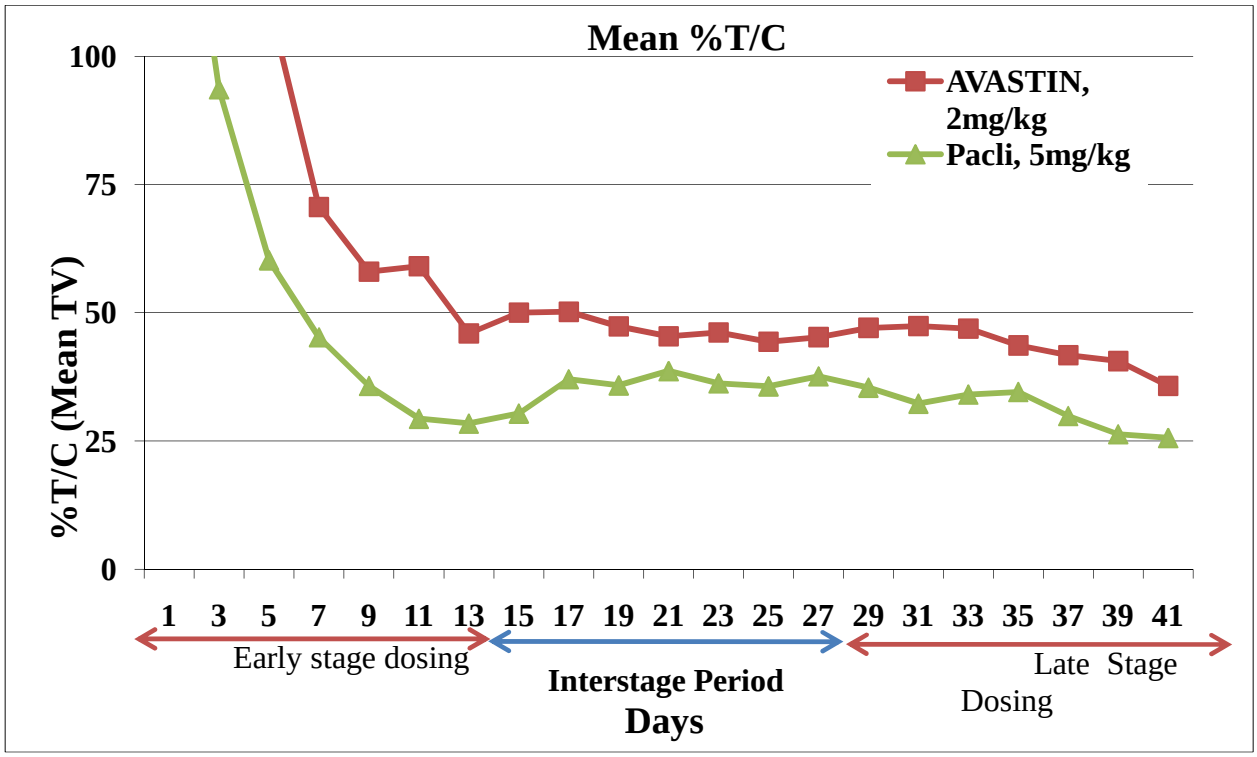

Fig.7: Mean % T/C Data.

	Days	Vehicle Control (G1)	Avastin, 2mg/kg (G2)	Paclitaxel, 5mg/kg (G3)
Early Stage Dosing	1	100.0	63.3	71.0
	3	100.0	54.5	42.3
	5	100.0	53.9	26.2
	7	100.0	30.6	5.6

	9	100.0	16.8	7.6
	11	100.0	13.6	2.3
	13	100.0	13.7	2.0
Inter stage period	15	100.0	17.7	4.5
	17	100.0	17.7	4.4
	19	100.0	17.1	4.6
	21	100.0	15.2	4.5
	23	100.0	14.8	0.1
	25	100.0	15.3	4.5
	27	100.0	16.1	4.6
Late Stage Dosing	29	100.0	17.5	5.4
	31	100.0	16.3	4.6
	33	100.0	15.9	4.1
	35	100.0	14.8	2.4
	37	100.0	13.1	3.6
	39	100.0	12.4	2.6
	41	100.0	11.4	1.5

Table: 9. - Median % T/C Data

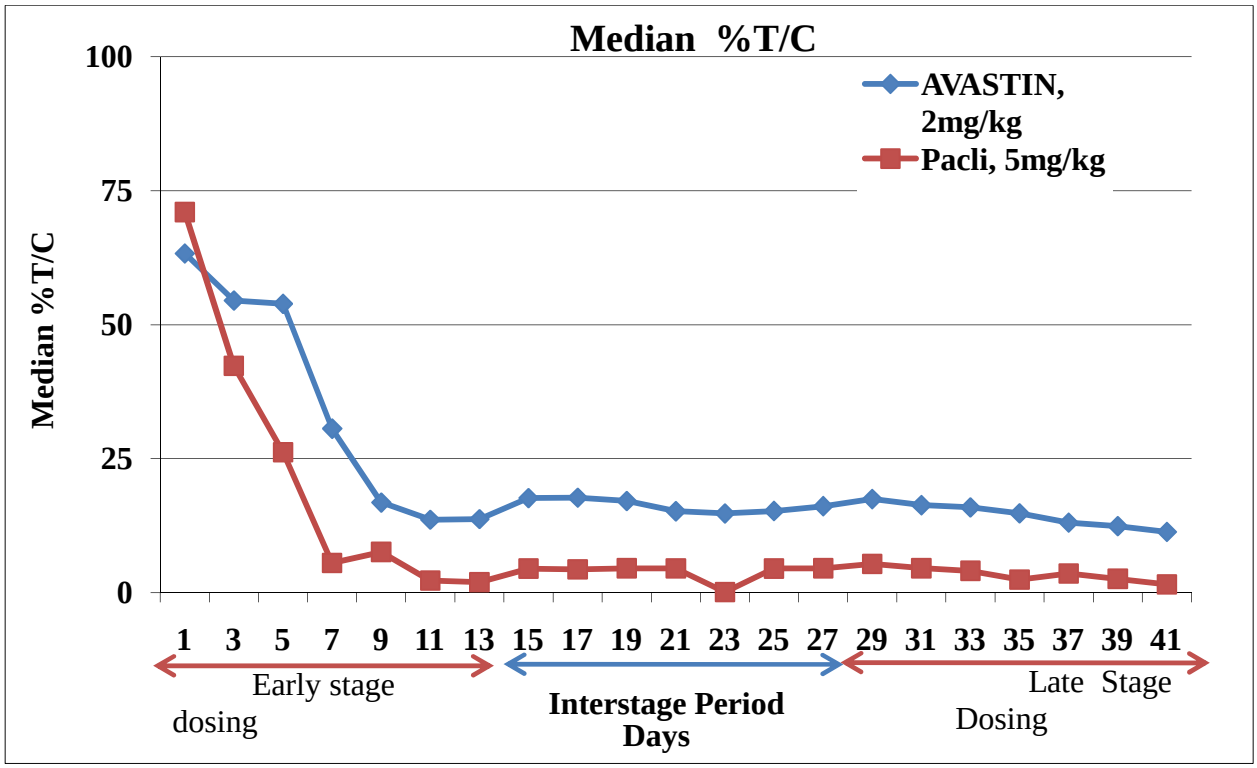

Fig 8:Median % T/C

5.2 Relative tumor volume (RTV)

Relative tumor volume for all the groups were calculated from median tumor volume data applying the following formula:-

$$\text{RTV at Day N} = \frac{\text{Median Tumor volume of Day N}}{\text{Median Tumor volume of Day 1}}$$

Data on relative tumor volume is represented in table 10 and fig 9.

	Days	Vehicle Control (G1)	Avastin, 2mg/kg (G2)	Paclitaxel, 5mg/kg (G3)
Early Stage Dosing	1	1.0	1.0	1.0
	3	2.5	2.1	1.5

	5	4.7	4.0	1.7
	7	8.9	4.3	0.7
	9	16.3	4.3	1.7
	11	26.8	5.8	0.9
	13	38.2	8.3	1.1
Inter stage period	15	43.4	12.1	2.8
	17	44.9	12.6	2.8
	19	48.6	13.1	3.1
	21	55.9	13.4	3.6
	23	61.1	14.3	0.1
	25	66.2	16.0	4.2
	27	69.9	17.8	4.5
Late Stage Dosing	29	70.5	19.5	5.3
	31	76.3	19.7	4.9
	33	80.1	20.2	4.6
	35	83.7	19.6	2.9
	37	92.2	19.1	4.7
	39	95.6	18.8	3.5
	41	102.0	18.3	2.2

Table: 10. – Relative Tumor Volume Data

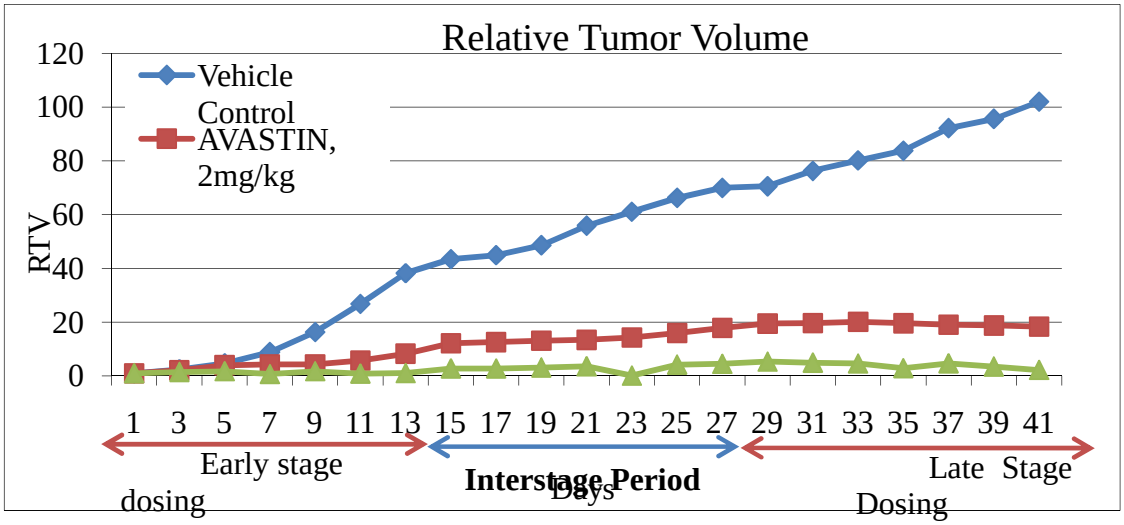

Fig:Relative Tumor Volume Data

Log Cell Kill (Lck)

Log cell kill for all the groups were calculated using TVDT of vehicle control group and time taken for target tumor volume data. Tumor target size for LCK calculation was taken as 1000 mm^3 and 5000 mm^3. Data on LCK is represented in table 11.

When tumor target size was taken 1000 mm^3, Avastin showed LCK value of 1.36, which is highly significant for its anti-cancer potential. Paclitaxel was not calculated as tumor volume did not reach up to 500 mm^3 throughout dosing period.

When tumor target size was taken 5000 mm^3, both drugs was unable to calculate as target size in both can't achieved.

DISCUSSION

The results of present research work demonstrate no clinical toxicity signs during cage side observation in any of the animal. None of the treatment group shows body weight loss >20%. Vehicle control and paclitaxel treatment showed two and one mortality respectively till end of experimental period. These mortalities are either incidental or due to increase in tumor burden. Intrestingly Avastin treatment did not show any mortality. All above findings support the non toxic nature of Avastin and Paclitaxel at given doses and regiment.

Paclitaxel 5mg/kg i.v on alternate day treatment for 5 times showed highest anticancer activity as it shown considerable tumor growth inhibition and did not allows the tumors to achieve the median tumor volume of 500 mm^3 throughout the study period. Avastin 2mg/kg i.v on alternate day also shows excellent anticancer activity however activity shown by Avastin was found not as much as Paclitaxel treatment.

For better understanding, the tumor volume data was expressed into different end points like % T/C, % tumors volume inhibition, relative tumor volume etc. Each parameter demonstrates promising anticancer activity of both tested drugs in both phases.

In early phase, Avastin 2mg/kg alternate day i.v. treatment for two weeks did not allow the tumor to reach 2000 mm^3, while in inter stage period where the drug treatment was stopped tumor volume was continuously increased. Same tumor growth pattern was observed in the Paclitaxel treatment group. In early phase, Paclitaxel 5mg/kg i.v on alternate day treatment for 5 times shows maximum tumor volume of 954.45 mm^3. As in inter stage drug treatment was stopped tumor volume of this group also persistently increased and reaches up to 2041 mm^3.

In lateral phase, both drug treatments were restarted to evaluate the effect of drugs on well developed tumors. Avastin treatment for two week in lateral phase was able to control the further growth of tumors. There was neither tumor regression nor tumour volume reduction observed due to Avastin treatment in lateral phase however tumor volume of this group was found stable throughout lateral phase treatment period. This clearly signify role of Avastin to restrict the further tumor growth in well developed tumors which indicate the cytostatic nature of the Avastin. Interestingly Paclitaxel treatment in lateral phase shows remarkable tumor growth suppression. In lateral phase Paclitaxel treatment not only controls the further tumor growth but also it found to be active in reduction of obtainable tumor volume. This reflects the ability of paclitaxel to kill the cells from tumors indicating its cytotoxic nature. Various researchers had been reported the cytotoxic and cytostatic nature of both drugs i.e. Avastin and paclitaxel against several solid tumors. Result of present experimental model is with accordance with that.

The lateral phase observations clearly distinguish between avastin and paclitaxel treatment. This above variation can be further explained by their mode of action.

Paclitaxel alters certain intracellular signal-transduction events, such as activation of mitogen-activated protein kinase and transcriptional activation of genes encoding a number of cytokines, such an interaction leads to cell cycle arrest and cytotoxicity. This may be one of the reason for paclitaxel, to show remarkable anticancer activity in early stage (where the tumors were in developing phase) as well as in late stage (where the tumors were well developed) where tumor reduction in treatment group was observed.

On other hand, Avastin which is a recombinant humanized monoclonal antibody that binds to and neutralizes the biologic activity of human vascular endothelial growth factor (VEGF). VEGF binding initiates angiogenesis. Avastin inhibits the binding of VEGF to its receptors, Flt-1 and KDR, on the surface of endothelial cells. therefore it prevents angiogenesis and hence suppresses tumor growth. In early stage (where the tumors were in developing phase) the avastin shows good anticancer effect pertaining to its anti-agiogenesis mechanisam however in late stage (where the tumors were well developed), avastin was only able to prevent further tumor growth and not able to reduced the tumour volume.

This above criterion was further well elucidated by relative tumor volume data. At the end of early phase, relative tumor volume shown by Avastin treatment group was found 8.3. In inter stage period the relative tumor volume of this group was consistently increased and it reaches to 17.8 on 27^{th} day. At lateral stage, when avastin treatment was restarted relative tumor volume of this group was between 18-20. Fascinatingly at the end of early phase, relative tumor volume shown by Paclitaxel treatment group was found 1.1. The relative tumor volume of this group was consistently increased and it reaches to 4.5 upto the end of inter stage period. At lateral stage, when paclitaxel treatment was restarted relative tumor volume of this group decline to 2.2

8. CONCLUSION

The present experimental design successfully provides a single model to screen anticancer compound in early stage as well as in late stge. Two standard anticancer drugs i.e. paclitaxel as well as Avastin were screened for their efficacy in BALB/c mice bearing CT-26 murine colon carcinoma. In early phase, Avastin 2mg/kg alternate day i.v. treatment for two weeks as well as Paclitaxel 5mg/kg i.v on alternate day treatment for 5 times shows remarkable

antitumor activity. In inter stage phase where the drug treatment were stopped for two weeks, tumor volume of both group animals showed persistence increment. In lateral phase, where drug treatment were restarted, in avastin treatment group, there was neither tumor regression nor tumor volume reduction was observed however tumor volume of this group was found stable throughout lateral phase. Interestingly, Paclitaxel treatment not only controls the further tumor growth but also it found to be active in reduction of obtainable tumor volume in lateral phase.

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