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bromide) which is metabolised in the mitochondria of metabolically active cells to a dark blue insoluble formazan product, SRB (Sulforhodamide B assay) which is a protein binding assay, and CV (crystal violet dye elution assay) which is a protein staining assay. At the end of the incubation periods the number of living cells were measured at an optical density of 570nm using a µQuant™ Biomolecular Spectrophotometer, MQX200, and Gen5™ Microplate Data Collection & Analysis software (BioTek® Instruments.Inc, April 2008, ®2006-2008, Revision E). Since absorbance measurements are influenced by many factors, such as sample turbidity, dust particles and bubbles, dirty microplates, well geometry and absorption to well surfaces, a second wavelength of absorbance for all the individual assays was studied in order to subtract the noise and deviations. In the MTT assay, the absorbance value at A^{570nm} was corrected by a second measurement at A^{630nm}. The same method was used for the SRB and CV assays, with an additional measurement at 690nm[1, 2, 3, 4].

Colorimetric – chemo-sensitivity assays

- For the MTT protocol, 20 µl of 5mg/ml MTT [bromide 3-(4,5-dimethyltio-azo-2)-2,5-diphanyl-tetrazole] (M2128, Sigma) was added to each well of plated cells which were then incubated for 3h at 37°C. At the end of the incubation period, the medium was removed and cells were rinsed with PBS (P3813, Sigma). The formazan crystals were dissolved with 100 µl of dimethyl-sulphoxide (DMSO) (D4540, Sigma)[5, 6, 7, 8, 9, 10, and 11].
- For the SRB assay, cells were fixed by layering 50 µl of 10% trichloroacetic acid (91228, Fluka,) and the plates were incubated at 4°C for 1h. After this period, cells were rinsed with water and stained with 100 µl of 0.4% SRB (341738, Sigma), dissolved in 1% acetic acid (401422, Carlo Erba), for 15 min. The unbound stain was removed, by washing twice with 1% acetic acid followed by the addition of 200 µl of 10mM Tris Buffer pH 10.5 (T6791, Sigma) in order to release the bound dye[12,13,14].
- For the CV assay, the medium was removed from the 96-well plates and each well was rinsed with PBS. Cells were fixed by adding 100 µl of 10% formalin (1.04003.2500, MERCK) for 20 min. The formalin was removed and then 100µl of 0.25% aqueous crystal violet (HT901, Sigma) was added for 10 min. Finally, by adding 100µl of 33% acetic acid, the stain or dye was dissolved[13, 15, 16, and 17].

Statistical Analysis

In order to have statistically acceptable results, all treatments for every cell line were conducted in triplicate, and were tested for the range of the dilutions mentioned above in comparison with untreated cells. The statistical significance of all effects was evaluated by a "difference of the means" test ($p< 0.05$).

Results

The results that have been generated indicate that there were some differences between colon and breast carcinomas.

Concerning colon cancer, it was observed that the population of cancer cells was obviously decreased when an extra amount of CV247 agent was added on the sixth day. The concentrations that had a greater effect were CV247 100µg/ml and CV247 200µg/ml combined with CDDP 50µg/ml. However, comparing the effect of the above two combinations,

Table 1: Statistic evaluation of absorbance values in HCT8 cell line

HCT8 cell line - Human Colon Adenocarcinoma

MEAN	MTT assay						SRB assay						CV assay					
	DAY 0	DAY 2	DAY 4	DAY 6	DAY 8	DAY 10	DAY 0	DAY 2	DAY 4	DAY 6	DAY 8	DAY 10	DAY 0	DAY 2	DAY 4	DAY 6	DAY 8	DAY 10
UNSTIMULATED CELLS	0,056	0,283	0,320	0,195	0,129	0,120	1,707	2,810	3,209	3,419	3,548	3,011	0,351	0,939	3,762	2,504	3,987	4,022
CV247100	0,035	0,196	0,339	0,306	0,253	0,250	1,858	2,798	3,023	3,147	3,024	2,971	0,397	0,830	2,189	3,330	3,018	4,021
CV247200	0,030	0,241	0,242	0,198	0,145	0,180	1,910	2,815	3,273	3,471	3,093	3,064	0,364	0,791	2,869	3,377	3,845	4,113
CP1	0,040	0,409	0,169	0,155	0,214	0,335	1,990	2,447	3,010	3,015	3,011	2,969	0,279	0,816	1,486	1,827	1,357	3,332
CP5	0,040	0,211	0,101	0,108	0,151	0,128	1,625	2,422	2,981	3,012	2,954	2,918	0,292	0,539	0,828	1,024	0,912	1,982
CP10	0,040	0,128	0,074	0,077	0,151	0,110	1,484	1,959	2,733	2,796	2,161	2,284	0,437	0,424	0,353	0,368	0,352	0,837
CP50	0,046	0,438	0,549	0,561	1,063	0,693	0,977	1,670	2,266	2,437	1,727	1,558	0,303	0,359	0,276	0,328	0,245	0,434
CV247100 + CP1	0,026	0,203	0,170	0,285	0,278	0,320	2,289	2,552	3,070	2,967	2,906	2,889	0,430	0,689	0,926	1,400	0,817	1,496
CV247100 + CP5	0,021	0,136	0,068	0,103	0,223	0,187	2,013	2,592	2,951	2,942	2,724	2,802	0,299	0,741	0,682	1,125	0,639	0,920
CV247100 + CP10	0,022	0,079	0,032	0,054	0,154	0,036	2,122	2,198	2,150	2,774	1,615	2,151	0,274	0,462	0,302	0,548	0,190	0,316
CV247100 + CP50	0,022	0,018	0,018	0,020	0,023	0,013	1,725	2,065	2,680	2,103	1,533	1,894	0,253	0,340	0,234	0,261	0,211	0,344
CV247 200 + CP1	0,031	0,220	0,146	0,285	0,250	0,275	2,174	2,756	3,048	3,010	2,969	2,909	0,430	0,942	1,281	1,950	0,800	2,262
CV247 200 + CP5	0,026	0,047	0,062	0,088	0,148	0,173	1,728	2,484	3,013	2,901	2,868	2,898	0,304	0,595	0,733	1,029	0,748	1,414
CV247 200 + CP10	0,029	0,097	0,034	0,048	0,047	0,033	2,064	2,295	2,810	2,495	1,586	2,159	0,274	0,445	0,306	0,346	0,198	0,506
CV247 200 + CP50	0,033	0,036	0,017	0,019	0,020	0,014	1,407	2,060	2,906	2,752	1,806	2,289	0,231	0,342	0,307	0,290	0,177	0,673
STD DEV	**DAY 0**	**DAY 2**	**DAY 4**	**DAY 6**	**DAY 8**	**DAY 10**	**DAY 0**	**DAY 2**	**DAY 4**	**DAY 6**	**DAY 8**	**DAY 10**	**DAY 0**	**DAY 2**	**DAY 4**	**DAY 6**	**DAY 8**	**DAY 10**
UNSTIMULATED CELLS	±0,014	±0,017	±0,033	±0,005	±0,020	±0,010	±0,240	±0,097	±0,131	±0,367	±0,029	±0,041	±0,020	±0,041	±0,050	±0,403	±0,525	±0,830
CV247100	±0,006	±0,033	±0,093	±0,010	±0,068	±0,035	±0,124	±0,158	±0,049	±0,122	±0,027	±0,012	±0,075	±0,044	±0,428	±0,408	±0,056	±0,415
CV247200	±0,002	±0,063	±0,017	±0,016	±0,018	±0,110	±0,432	±0,135	±0,183	±0,157	±0,002	±0,034	±0,103	±0,158	±0,042	±0,540	±0,108	±0,253
CP1	±0,008	±0,048	±0,007	±0,018	±0,020	±0,117	±0,162	±0,306	±0,093	±0,018	±0,015	±0,012	±0,074	±0,087	±0,101	±0,527	±0,548	±0,334
CP5	±0,009	±0,037	±0,021	±0,006	±0,037	±0,029	±0,100	±0,480	±0,027	±0,096	±0,013	±0,026	±0,031	±0,175	±0,038	±0,154	±0,033	±0,240
CP10	±0,003	±0,022	±0,024	±0,009	±0,041	±0,016	±0,098	±0,080	±0,027	±0,103	±0,183	±0,219	±0,122	±0,095	±0,016	±0,116	±0,064	±0,073
CP50	±0,005	±0,400	±0,742	±0,558	±0,909	±0,440	±0,055	±0,126	±0,377	±0,427	±0,666	±0,327	±0,083	±0,023	±0,032	±0,101	±0,067	±0,175
CV247100 + CP1	±0,003	±0,060	±0,002	±0,071	±0,044	±0,187	±0,302	±0,088	±0,040	±0,045	±0,026	±0,006	±0,089	±0,048	±0,043	±0,162	±0,284	±0,131
CV247100 + CP5	±0,001	±0,015	±0,031	±0,026	±0,012	±0,024	±0,151	±0,256	±0,022	±0,078	±0,071	±0,016	±0,072	±0,205	±0,168	±0,408	±0,184	±0,255
CV247100 + CP10	±0,001	±0,017	±0,010	±0,019	±0,146	±0,017	±0,206	±0,136	±0,125	±0,045	±0,286	±0,211	±0,042	±0,045	±0,045	±0,198	±0,019	±0,096
CV247100 + CP50	±0,002	±0,008	±0,005	±0,003	±0,007	±0,003	±0,199	±0,212	±0,196	±0,190	±0,127	±0,153	±0,026	±0,118	±0,065	±0,065	±0,008	±0,139
CV247 200 + CP1	±0,001	±0,045	±0,007	±0,027	±0,022	±0,041	±0,120	±0,111	±0,038	±0,096	±0,003	±0,008	±0,116	±0,108	±0,063	±0,265	±0,317	±0,221
CV247 200 + CP5	±0,003	±0,006	±0,016	±0,014	±0,021	±0,059	±0,118	±0,116	±0,056	±0,055	±0,034	±0,059	±0,099	±0,023	±0,047	±0,039	±0,008	±0,126
CV247 200 + CP10	±0,003	±0,013	±0,003	±0,006	±0,006	±0,010	±0,230	±0,249	±0,130	±0,225	±0,031	±0,313	±0,090	±0,061	±0,022	±0,039	±0,022	±0,037
CV247 200 + CP50	±0,005	±0,003	±0,006	±0,001	±0,003	±0,002	±0,041	±0,220	±0,025	±0,064	±0,262	±0,305	±0,090	±0,031	±0,083	±0,055	±0,020	±0,360

Table 2: Statistic evaluation of decrease fold values in HCT8 cell line

DECREASE FOLD	MTT assay						SRB assay						CV assay					
	DAY 0	DAY 2	DAY 4	DAY 6	DAY 8	DAY 10	DAY 0	DAY 2	DAY 4	DAY 6	DAY 8	DAY 10	DAY 0	DAY 2	DAY 4	DAY 6	DAY 8	DAY 10
CV247100	37,5	30,7	-5,9	-56,9	-96,1	-108,3	-6,8	0,4	5,8	8,0	0,8	1,3	-13,1	11,6	41,8	-33,0	24,3	0,0
CV247200	46,4	14,8	24,4	-1,5	-12,4	-50,0	-11,9	-0,2	-2,0	-1,5	-1,5	-2,4	-0,9	-125,4	-717,4	-862,1	-995,4	-1071,8
CP1	28,6	-44,5	47,2	20,5	-85,9	-179,2	-16,6	12,9	6,2	11,8	1,2	1,4	20,5	13,1	60,5	27,0	66,0	17,2
CP5	28,6	25,4	68,4	44,6	-17,1	-6,7	4,8	13,8	7,1	11,9	3,1	3,1	16,8	42,6	78,0	59,1	77,1	90,7
CP10	28,6	54,8	78,9	60,5	-17,1	8,3	13,1	30,3	14,8	18,2	29,1	24,1	-24,5	54,8	90,6	84,5	91,2	79,2
CP50	17,9	-54,8	-71,6	-187,7	-724,0	-477,5	42,8	40,6	29,4	28,7	43,3	48,3	13,7	61,8	92,7	86,9	93,9	89,2
CV247100 + CP1	53,6	26,3	46,9	-46,2	-115,5	-166,7	-34,1	9,3	4,3	13,2	4,7	4,1	-22,5	36,6	75,4	44,1	79,5	62,6
CV247100 + CP5	62,5	51,9	78,8	47,2	-72,9	10,8	-17,9	7,8	8,0	14,0	10,6	6,8	14,8	21,1	81,9	55,1	84,0	77,1
CV247100 + CP10	60,7	72,1	90,0	72,3	-19,4	70,0	-24,3	21,8	33,0	18,9	47,0	28,6	21,9	50,8	92,0	78,1	95,2	92,1
CV247100 + CP50	60,7	93,6	94,4	89,7	82,2	89,2	-1,1	26,5	16,5	38,5	49,7	37,1	27,9	63,6	93,8	89,6	94,7	91,4
CV247200 + CP1	44,8	22,3	54,4	-46,2	-93,8	-129,2	-27,4	1,9	5,8	12,0	2,8	3,4	-22,5	-168,4	-265,0	-455,6	-127,9	-544,6
CV247200 + CP5	53,6	83,4	80,8	54,9	-14,7	-44,2	-1,2	11,6	6,1	15,2	5,9	3,8	13,4	-89,5	-108,9	-193,2	-113,1	-302,8
CV247200 + CP10	48,2	65,7	89,4	75,4	63,6	72,5	-20,9	18,3	12,4	27,0	48,0	28,3	21,9	-26,8	12,8	1,4	43,6	-44,2
CV247200 + CP50	41,1	67,3	94,7	90,3	84,5	88,3	17,6	26,7	9,4	19,2	40,7	24,0	34,2	2,6	12,5	17,4	49,6	-91,7

Table 3: Statistic evaluation of absorbance values in MDA-MB 231 cell line

MDA-MB 231 cell line - Human Breast Adenocarcinoma

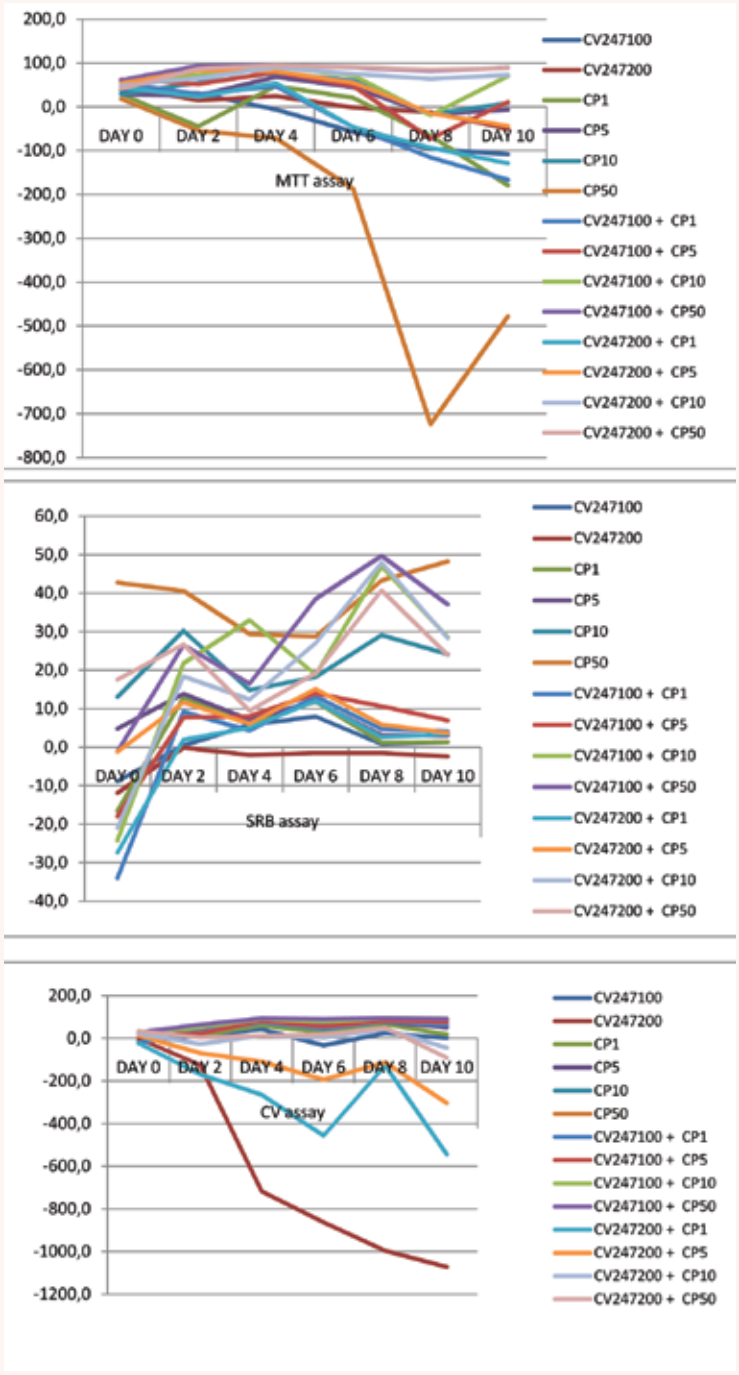
MEAN	MTT assay						SRB assay						CV assay					
	DAY 0	DAY 2	DAY 4	DAY 6	DAY 8	DAY 10	DAY 0	DAY 2	DAY 4	DAY 6	DAY 8	DAY 10	DAY 0	DAY 2	DAY 4	DAY 6	DAY 8	DAY 10
UNSTIMULATED CELLS	0,010	0,057	0,058	0,294	0,257	0,273	1,571	1,753	2,583	2,889	3,018	2,966	0,225	0,320	0,501	1,791	1,353	3,554
CV247100	0,010	0,269	0,145	0,181	0,149	0,173	1,819	1,732	2,695	2,815	2,244	2,432	0,263	0,227	0,222	0,202	0,149	0,263
CV247200	0,009	0,020	0,013	0,023	0,017	0,060	1,838	2,157	2,276	2,083	2,769	1,856	0,194	0,297	0,301	0,351	0,219	0,312
CP1	0,010	0,031	0,046	0,157	0,170	0,186	1,869	2,139	2,752	2,892	2,995	2,951	0,164	0,348	0,841	1,905	1,181	3,616
CP5	0,008	0,031	0,042	0,132	0,159	0,146	2,004	1,906	2,610	2,758	2,945	2,913	0,152	0,340	0,652	1,057	0,808	2,751
CP10	0,008	0,029	0,089	0,091	0,039	0,027	1,720	2,146	2,546	2,175	2,564	2,240	0,162	0,354	0,475	0,422	0,188	0,537
CP50	0,008	0,019	0,019	0,153	0,013	0,005	1,536	1,642	2,087	1,721	2,335	1,448	0,188	0,225	0,257	0,247	0,094	0,226
CV247100 + CP1	0,007	0,020	0,016	0,009	0,008	0,005	1,536	1,642	2,087	1,721	2,335	1,448	0,365	0,222	0,238	0,169	0,008	0,156
CV247100 + CP5	0,004	0,014	0,007	0,005	0,007	0,005	1,709	1,769	2,061	1,596	2,089	1,468	0,212	0,168	0,215	0,191	0,007	0,527
CV247100 + CP10	0,005	0,012	0,005	0,005	0,006	0,005	1,926	1,872	1,968	1,616	2,143	1,466	0,174	0,209	0,123	0,141	0,006	0,207
CV247100 + CP50	0,006	0,008	0,006	0,003	0,002	0,004	1,811	1,393	1,840	1,640	2,143	1,621	0,163	0,158	0,117	0,118	0,002	0,139
CV247 200 + CP1	0,009	0,017	0,012	0,022	0,016	0,016	2,033	2,363	2,369	2,241	2,558	2,006	0,247	0,337	0,323	0,296	0,118	0,186
CV247 200 + CP5	0,007	0,019	0,016	0,028	0,020	0,016	1,784	2,157	2,465	2,202	2,287	1,662	0,187	0,273	0,340	0,377	0,269	0,304
CV247 200 + CP10	0,006	0,019	0,014	0,026	0,025	0,012	1,646	2,042	2,182	2,087	2,142	1,970	0,177	0,241	0,378	0,357	0,275	0,314
CV247 200 + CP50	0,005	0,008	0,006	0,007	0,005	0,004	1,658	1,838	1,911	1,735	1,856	1,357	0,143	0,199	0,166	0,130	0,087	0,130
STD DEV	**DAY 0**	**DAY 2**	**DAY 4**	**DAY 6**	**DAY 8**	**DAY 10**	**DAY 0**	**DAY 2**	**DAY 4**	**DAY 6**	**DAY 8**	**DAY 10**	**DAY 0**	**DAY 2**	**DAY 4**	**DAY 6**	**DAY 8**	**DAY 10**
UNSTIMULATED CELLS	±0,003	±0,008	±0,009	±0,265	±0,028	±0,047	±0,152	±0,108	±0,139	±0,013	±0,012	±0,021	±0,070	±0,024	±0,031	±0,157	±0,079	±0,150
CV247100	±0,003	±0,092	±0,026	±0,036	±0,032	±0,041	±0,105	±0,213	±0,056	±0,146	±0,165	±0,368	±0,075	±0,006	±0,046	±0,013	±0,032	±0,065
CV247200	±0,003	±0,009	±0,004	±0,003	±0,007	±0,125	±0,108	±0,224	±0,304	±0,174	±0,060	±0,131	±0,046	±0,039	±0,003	±0,017	±0,002	±0,015
CP1	±0,004	±0,007	±0,007	±0,073	±0,026	±0,041	±0,328	±0,132	±0,043	±0,005	±0,018	±0,010	±0,006	±0,029	±0,027	±0,104	±0,054	±0,132
CP5	±0,001	±0,010	±0,007	±0,052	±0,038	±0,025	±0,164	±0,240	±0,086	±0,028	±0,012	±0,010	±0,007	±0,014	±0,046	±0,123	±0,053	±0,100
CP10	±0,001	±0,003	±0,057	±0,067	±0,010	±0,007	±0,274	±0,362	±0,011	±0,116	±0,079	±0,184	±0,017	±0,024	±0,025	±0,017	±0,026	±0,050
CP50	±0,002	±0,003	±0,055	±0,203	±0,002	±0,001	±0,080	±0,165	±0,176	±0,039	±0,215	±0,159	±0,028	±0,040	±0,016	±0,030	±0,013	±0,014
CV247100 + CP1	±0,001	±0,018	±0,003	±0,005	±0,006	±0,008	±0,159	±0,362	±0,064	±0,147	±0,283	±0,032	±0,154	±0,026	±0,026	±0,019	±0,006	±0,007
CV247100 + CP5	±0,001	±0,001	±0,002	±0,002	±0,003	±0,002	±0,084	±0,239	±0,282	±0,123	±0,165	±0,172	±0,018	±0,025	±0,046	±0,095	±0,003	±0,556
CV247100 + CP10	±0,002	±0,001	±0,001	±0,002	±0,003	±0,003	±0,321	±0,258	±0,065	±0,033	±0,192	±0,184	±0,021	±0,005	±0,006	±0,036	±0,003	±0,094
CV247100 + CP50	±0,000	±0,002	±0,003	±0,002	±0,001	±0,002	±0,087	±0,082	±0,190	±0,124	±0,223	±0,143	±0,010	±0,006	±0,014	±0,014	±0,001	±0,018
CV247 200 + CP1	±0,003	±0,006	±0,002	±0,004	±0,009	±0,010	±0,065	±0,183	±0,253	±0,164	±0,055	±0,277	±0,037	±0,062	±0,031	±0,042	±0,007	±0,025
CV247 200 + CP5	±0,002	±0,003	±0,005	±0,002	±0,011	±0,010	±0,305	±0,195	±0,238	±0,272	±0,129	±0,242	±0,042	±0,039	±0,042	±0,124	±0,150	±0,085
CV247 200 + CP10	±0,003	±0,011	±0,001	±0,005	±0,014	±0,002	±0,237	±0,096	±0,059	±0,017	±0,083	±0,155	±0,007	±0,013	±0,052	±0,074	±0,038	±0,012
CV247 200 + CP50	±0,001	±0,002	±0,002	±0,004	±0,003	±0,001	±0,044	±0,214	±0,251	±0,156	±0,168	±0,048	±0,015	±0,012	±0,040	±0,035	±0,002	±0,012

the CV247 100μg/ml combined with CDDP 50μg/ml had more acceptable results in all three assays performed (MTT, SRB and CV assay). The decrease in HCT8, human colon adenocarcinoma, ranged from 82% to 94% (MTT assay), 37% to 49.7% (SRB assay) and 89.5% to 94.7% (CV assay) on the sixth day. To conclude, there was an obvious decrease rate of the percentage of colon cancerous cells when these were incubated with CV247 100μg/ml combined with CDDP 50μg/ml, on the sixth day of the incubation period (see Tables 1-2 and Figure 1).

In the breast cancer cell lines that were tested, it was observed that the extra amount of CV247 component that was added in the sixth day of the incubation period had no positive impact in the decrease of the cancerous cells. There was an obvious decrease rate of the cells when treated with the combination of CV247 and CDDP in all their different concentrations. However, the optimum concentration that had decreased the breast cancer cells was CV247 100μg/ml combined with CDDP 50μg/ml during the eighth day of the incubation period. See Tables 3-4 and Figure 2.

In order to calculate the decrease fold, the absorbance measurements have been used. The absorbance is given by the Beer-Lambert law where the formula is A=Ecl, (where A is absorbance, E is the extinction coefficient, l is the distance the light travels through the material, and c is the concentration of the absorbing species within the material[18].

Figure 1: Decrease fold values in HCT8 cell line



Discussion

During the last year, it has been observed an increased number of patients who suffer from many types of cancer. The need to prevent, treat as well as to improve the standard of living of these patients is imperative.

For this purpose many compounds have been established. CDDP is a platinum-based chemotherapy drug that is used to treat various types of cancer but basically sarcomas and ovarian carcinomas[19,20,21,22]. Its biochemical mechanism involves the binding of the drug to DNA and non- DNA targets and the subsequent induction of cell death through apoptosis and necrosis. This molecular model is regulated by the intracellular redox, potential generated by the pyridine nucleotide pool (NAD^+/NADH and $NADP^+$/NADPH) as well as by the free cellular energy available from the ATP/ADP ratio. It is generally acceptable that when CDDP binds to genomic DNA (gDNA) into the cell nucleus, is mostly responsible for its antitumor properties.[23].

Although CDDP is a widely used chemotherapeutic agent, it causes considerably adverse side effects when it is used alone in a chemotherapeutic model. At first, it causes high toxicity (nephrotoxicity, neurotoxicity, ototoxicity, electrolyte disturbance, nausea and vomiting) so there is a dose-limiting factor which reduces its healing properties[24,25,26]. On the other hand, the most common hallmark characterizes the majority of anticancer agents, including CDDP, is the drug resistance pre and/or after treatment. In comparison with other cancer types (such as head, neck, testicular or ovarian carcinomas) colorectal and breast tumors are resistant to CDDP therapy exhibiting several resistant pathways acting simultaneously in order for cancer cells to escape cell death[24, 27, 28, 29, 30 & 31].

Another substance that has been used as a chemotherapeutic agent and has been proved to be cytotoxic-cytostatic against colon and breast tumors, is the CV247 compound. It consists of four already known substances: gluconate manganese, gluconate copper, ascorbic acid (vitamin C) and sodium salycylate (SS), and improves symptoms as well as prolonging life in patients with terminal cancer types by stimulating the immune system or by stimulating or down-regulating the production of cytokines such as IL-18[32, 33].

According to previous studies, CDDP has a synergist effect when combined with other anti-cancer drugs. The purpose of this study was to evaluate the efficacy of CDDP and CV247 separately as well as in combination, in three human colon cancer cell lines (LOVO, HCT8, HT55) and in three human breast cancer cell lines (MDA-MB 231, T47D, MFM-223) over a time period of a ten-day assay. According to the results, there is clear evidence that the combination of CDDP and CV247 decreases dramatically the percentage of both colon and breast cancer cells when compared with the effect of each substance separately. This suggests that the doses are time- and concentrate-dependant, and if the therapeutic regimen will be administered over a more extensive time period (ten days) and in relatively low concentrations, it will reduce the negative consequences of CDDP and CV247 separately [34, 35, 36, 37, 38, 39, 40, 41, 42].

Conclusion

In conclusion, this study enhances the cytotoxic-cytostatic effect of CDDP and CV247 in colon and breast carcinomas in a time period of ten days. It was found that the CDDP and CV247 combination was able to decrease the number of viable cancer cells by up to 80 % of the total cell population on the sixth day of the incubation period, particularly in human colon cancer cell lines when compared to untreated cell lines. Further study is warranted to determine the efficacy of this combination regimen in a greater spectrum of cell lines.

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Maria Toloudi studied molecular biology and genetics in the University of Thrace and now I have been working for about three years in the R.G.C.C as a member of the research and development department. I am active especially in the field of cellular and molecular biology dealing with human cancer stem cells.
Email: toloudi.maria@rgcc-genlab.com

Panagiotis Apostolou graduated from the Department of Molecular Biology and Genetics, Democritus University of Thrace, in 2007. Since October 2008 I have been working in the research and development department of the R.G.C.C. (Research Genetic Cancer Center Ltd, www.rgcc-genlab.com), and I am active in the field of molecular biology. **Email: apostolou.panagiotis@rgcc-genlab.com**

Marina Chatziioannou was born and grew up in Greece. I studied biochemistry and biotechnology at the University of Thessaly (2001-2006). I have worked at the R.G.C.C. laboratory since 2006, at the clinical department, flow cytometry section. I mainly work on Human Circulating Tumour Cells (CTCs).
Email: chatzioannou.marina@rgcc-genlab.com

Dr Roger Oakes is a biochemist with over 35 years experience in drug development.
Email: roger.oakes47@btinternet.com

Ioannis Papasotiriou was born in Germany in 1973 and returned in childhood to Greece, where I studied in the Medical School of Thessaloniki, specialising in human genetics in Switzerland. Two master degree awards have been obtained in molecular biology in medicine from Westminster University (UK), and in oncology from the University of Nottingham (UK). I was promoted to MD in MLU in Germany under the field of evaluation of TKIs in human cancer cell lines. Since 2004 I have been the director and founder of RGCC Ltd, which is active in both areas of service (research and clinical).
Email: papasotiriou.ioannis@rgcc-genlab.com

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Bringing Proven Clinical Trial Electronic Payment Solutions to Emerging Markets

PEER REVIEWED

North America is the largest market for CROs, with over 36,281 sites having completed clinical trials and actively recruiting in the United States, and 3032 in Canada.[1] Western Europe has the second largest share of clinical trial sites across its numerous countries. As such, these two regions have long been considered the traditional markets for clinical research organisations (CROs). However, emerging markets are presenting significant potential for the expansion of clinical trial services.

The various emerging markets that are being identified as huge potential growth areas each have unique benefits with regards to regulatory landscapes, potential patient population, patient access, recruitment and retention, and treatment naivety. However, the emerging markets also present substantial challenges that can impede clinical trial progress and sometimes even threaten the overall success of a trial. Clinical trial payments represent one of the challenges CROs, investigators and sites face in both traditional and emerging markets. Approximately 20 % of clinical trial sites worldwide are located in countries which have relatively poor banking infrastructures compared to North America and Europe.[2] This can make obtaining payments via traditional methods difficult for subjects in emerging markets due to lack of bank accounts or large administration fees.

Payments to clinical trial subjects are often a topic of hot debate, and are generally considered either a primary motivator for patient participation or at least a measure of fair subject compensation for time, risk, and travel expenses. CROs can differ substantially in the marketing tactics they use to recruit subjects for clinical trials, based on these perceived patient priorities.

With regards to subject payment, CRO Parexel states that "**it is therefore considered ethical and appropriate that you should be paid for your time and inconvenience**"[3] and therefore does not push payment as a principal benefit of clinical trial participation, but rather as a necessary and ethical obligation. Conversely, Covance attempts to generate interest by mentioning financial reimbursement ahead of medical advancements: "By volunteering for medical trials with Covance, everyone from students to office workers to retired couples are being paid up to £2,500 and helping to make a real difference to pioneering medical breakthroughs."[4]

While patient payments have been an integral element in clinical trials for decades, both in established and emerging clinical trials markets, the technology to efficiently manage and deliver payments has remained a technological backwater, dominated by outdated manual processes. It is estimated that almost 1.3 million people worldwide participate in clinical trials at over 95,000 sites each year, meaning that eight million payments are made by CROs, clinical research sites and investigators to patients.[5] Traditionally the majority of these payments have been made using cheques, which have been reported to incur significant administration costs at both the site and sponsor levels, and can involve more than four manual steps to administer a single patient payment. These steps can also require review and approval by multiple departments at both the site and the sponsor.[6]

Payments in cash, although flexible, can also present significant problems to clinical trial subjects, with obvious security issues associated with carrying substantial amounts of cash. In addition to issues of patient safety, cash payments also present problems to CROs, sites and investigators as cash payments require extensive manual tracking and monitoring. The multiple drawbacks associated with traditional payment methods highlight the need for more time-efficient, cost-effective and patient-friendly solutions to reimburse patients taking part in clinical trials.

Patient retention and recruitment are often judged as two of the most important factors in a successful clinical trial.[7] Statistics show that delays associated with missed recruitment targets can dramatically increase the cost of conducting the trial.[8] Around 20-30 % of patients drop out of Phase II/III trails which can be, in part, due to the lack of a robust and cost-effective patient payment solution.[9] Electronic payment solutions, such as the ClinCard system from Greenphire, present subjects with a replacement for traditional cheque-based and cash payment methods while providing additional benefits to help improve the patient experience.

Reduced Site and Sponsor Administration

Clinical trials using electronic payment solutions provide trial subjects with a prepaid debit card. Payment management is handled through a web-based platform which enables the centralisation of payments. Payments are made to the subject via the debit card which can be PIN-protected, FDIC insured and subject to consumer protection regulations. In comparison to traditional methods such as cheque or cash payment, prepaid cards dramatically reduce site and sponsor administration. The company does not have to cut, produce and mail hundreds of cheques to hundreds of individuals, saving on administrative costs associated with these manual procedures.

Efficient Approval and Reporting Processes

Through a centralised system, electronic payment solutions offer an efficient electronic approval system to ensure the payment process runs as accurately and efficiently as possible. Sponsors have the option of controlling and monitoring release of payments to patients, or delegating this responsibility to site administrators.