Activated Cancer Therapy Using Light and Ultrasound - A Case Series of Sonodynamic Photodynamic Therapy in 115 Patients over a 4 Year Period

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Abstract: Activated Cancer Therapy (ACT), also known as Sonodynamic Photodynamic Therapy (SPDT) is a novel therapeutic modality that utilises a non-toxic photosensitive agent with reported ultrasound-activated properties. SPDT has previously demonstrated significant tumour cell inhibition in animal studies. There has been much research into the efficacy of photodynamic therapy and development in understanding of the underlying mechanism of tumour cytotoxicity. Synergistic ultrasound activation represents a promising development to activated sensitiser therapy, as photo-activation is limited by access and penetrance issues. Ultrasound has been demonstrated to activate a number of sono-sensitive agents allowing the possibility of non-invasive targeted treatment of deeper tumour sites than is currently achievable with photodynamic therapy. This case series of 115 patients with a variety of cancer diagnoses reports on experiences of this treatment over a 4 year period using sublingual administration of a new dual activation agent, Sonnelux-1, followed by a protocol of LED light and low-intensity ultrasound exposure. Initial clinical observation suggests SPDT is worthy of further investigation as an effective and well tolerated treatment for a wide variety of primary and metastatic tumours, including those refractory to chemotherapy.

Key Words: Sonodynamic therapy, photodynamic therapy, activated cancer therapy, ultrasound activated therapy, metastatic cancer, sonnelux-1, dove clinic, sonnemed.

INTRODUCTION

This case series of 115 patients with a variety of cancer diagnoses outlines clinical outcomes over a 4 year period of Activated Cancer Therapy (ACT) also known as Sonodynamic Photodynamic Therapy (SPDT) or the Sonnelux Protocol. This is a novel therapeutic modality that utilises a non-toxic photosensitive agent with reported ultrasound-activated properties. This treatment centres around the development of a specific light and ultrasound activated sensitiser (Sonnelux-1) which has previously demonstrated tumour cell inhibition in animal studies, and may provide a new method of inducing targeted tumour cell necrosis. Many of the patients included in this case series have advanced metastatic cancer diagnoses, and many have failed to respond to conventional management approaches. Numerous cases are reported showing significant extension of predicted median survival with reduced tumour mass and stable disease both clinically and on imaging.

BACKGROUND: ACTIVATED CANCER THERAPY

1. Photodynamic Therapy – Light Activated Therapy

Photodynamic Therapy (PDT) is an established therapeutic option for a variety of pre-cancerous and malignant pathologies [1-5]. The majority of PDT photosensitive agents possess a heterocyclic ring structure similar to that of chlorophyll or the haem group in haemoglobin [6], that can be administered *via* topical or systemic routes. The photosensitiser becomes activated by light energy applied from an LED or coherent laser emission source.

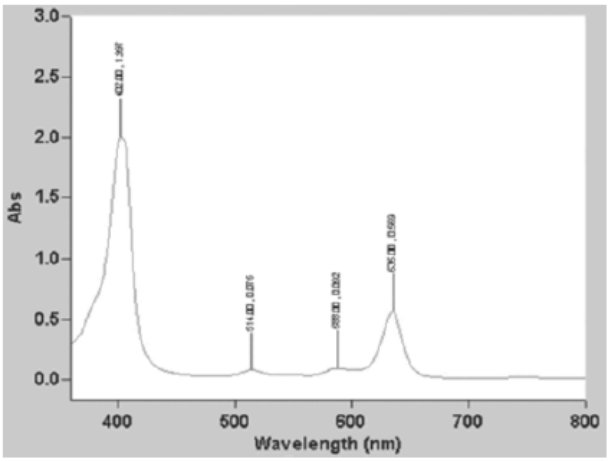

Fig. (1). A graph to show light absorption by Sonnelux-1 by specific wavelength (Sonnelux-1 diluted 1: 1000). Absorption scan, "Chem Lab" instrument.

Following absorption of light at a specific wavelength by the photosensitiser, a transfer and translation occurs of light energy into a chemical reaction. In the presence of molecular oxygen this produces singlet oxygen ($^1O_2$) or superoxide (O_2^-), and induces cell damage through direct and indirect cytotoxicity [6].

A variety of photosensitisers demonstrate selective absorption into malignant cells, increasing the potential to target cytotoxicity [6, 7] and limit unwanted side-effects.

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Photo-activation is however limited to surface pathology, or tumour mass capable of being targeted *via* endoscopic access. This is due to absorption of light into surrounding tissue, which creates limitation on penetrance and the depth of photosensitiser activation. The use of new photosensitisers sensitive to longer wavelengths of light increases depth of penetration [8], but effective non-invasive treatment of deep tumour sites remains problematic.

2. Sonodynamic Therapy – Ultrasound Activated Therapy

Ultrasound is a mechanical wave with periodic vibrations of particles in a continuous, elastic medium at frequencies equal to or greater than 20 kHz. It is not only perceived as safe, but has excellent tissue penetrating ability without major attenuation of its energy [9, 10]. Therefore the potential medical application of ultrasound has been evaluated extensively and has lead to the routine use of ultrasound for diagnostic imaging of soft tissue [11].

Ultrasound Activated Therapy (sonodynamic therapy), the ultrasound dependent enhancement of cytotoxic activities of certain compounds (sonosensitisers), is an attractive modality for cancer treatment with potential to focus the ultrasound energy on tumour sites buried deep in tissues and to locally activate a preloaded sonosensitiser.

The effect can be localised by focusing the ultrasound on a defined region and choosing compounds with tumour affinity [12-14], causing enhanced cytotoxicity at pathological sites with minimal damage to peripheral healthy tissue.

Potentiated cytotoxicity by ultrasound was first demonstrated when mouse leukemia L1210 cells were exposed to continuous wave ultrasound (2 MHz, 10 W/cm2) while suspended in nitrogen mustard solution *in vitro*. Mice subsequently inoculated with these cells had longer survival times than control animals that received cells exposed to the drug but not ultrasound [15].

Following this, the application of low-level ultrasound to a biological target was found to potentiate chemotherapeutic cell killing with adriamycin and diaziquone [16]. *In vivo*, this combined drug and ultrasound treatment resulted in statistically significant reductions in tumor volume of uterine cervical squamous cell carcinoma implanted in the cheek pouch of the Syrian hamster compared to the chemotherapeutic alone. The ultrasound applied without the chemotherapy agent was non-cytotoxic and produced negligible temperature elevation.

The photodynamic sensitisers have also been studied for ultrasound-activated properties. They have the benefit of being non-toxic unless activated and have been demonstrated to have tumour localizing properties [6, 7]. Hematoporphyrin, a commonly used photo-sensitiser enhanced the killing of mouse sarcoma and rat ascites 130 tumor cells exposed *in vitro* to ultrasound (1.92 MHz) at intensities of 1.27 and 3.18 w/cm2, from 30% and 50% to 99% to 95% respectively [17].

Possible cytotoxic mechanisms include generation of sonosensitiser-derived radicals which initiate chain peroxidation of membrane lipids *via* peroxyl and/or alkoxyl radicals, the physical destabilization of the cell membrane by the sonosensitizer thereby rendering the cell more susceptible to shear forces and cavitation effects or ultrasound enhanced drug transport across the cell membrane (sonoporation) [14, 18, 19].

SONNELUX -1 – A DUAL ACTIVATION AGENT

Light and Ultrasound Activation

Sonnelux-1 is a metallo-chlorin complex, containing a highly purified mixture of several chlorophyllins, each with a different side chain and an average molecular weight of 942. Sonnelux-1 has photo-activation properties and has also been demonstrated to be extremely sensitive to ultrasound [20].

Safety studies, including LC50 studies of Sonnelux-1 as determined in zebrafish, reveal that Sonnelux-1 is essentially non-toxic. No zebrafish death is noted at the maximum soluble concentration of the sonosensitiser (data pending publication). 7 Sonnelux-1 is registered as non-hazardous according to OSHA standards and EU directives.

Sonnelux-1 Animal Studies Demonstrating Dose-Dependent Ultrasound Activated Tumour Cytotoxicity

Sonnelux-1 has demonstrated significant tumour cell cytotoxicity following ultrasound-activation using a mouse S-180 Sarcoma model [21]. Following treatment, tumour volume was monitored. Significant tumour growth inhibition was seen in the group that was administered both ultrasound and Sonnelux-1 with significant ($p<0.01$) reduction in mean tumour weight (see Fig. **2**). No significant difference occurred with ultrasound or sonnelux administration alone.

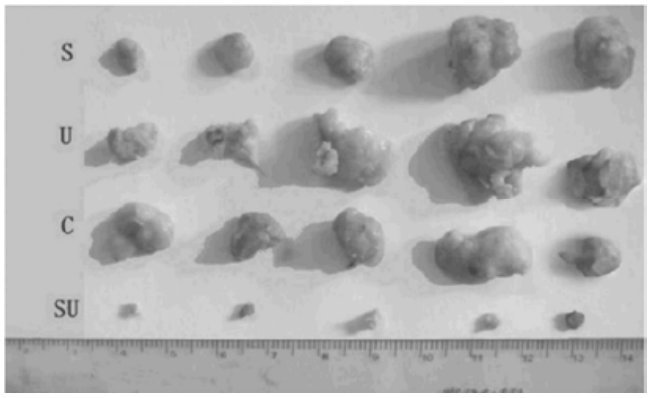


Fig. (2). Photographs of mouse S-180 tumours peeled off 15 days after treatment from each group of mice, showing significant reduction in tumour volume after combined sonnelux-1 and ultrasound administration in a light tight room. Top line (S) – Sonnelux-1 treatment without ultrasound or light exposure. Second line (U) - ultrasound 1. 2W/cm2 without Sonnelux-1 administration. Third line (C) – Control sample without ultrasound or Sonnelux-1 administration. Fourth line (SU) - Sonnelux 1 treatment plus ultrasound 1.2W/cm2 in a light tight room.

Significantly, cytotoxicity increased in a dose-dependent manner from 0.3W/cm2 to 1.2W/cm2 (see Fig. **3**). Histology showed coagulated necrosis or metamorphic tissue which started within 2 hours of ultrasound activation [21].

Tumour cytotoxicity was also reported when a bone-barrier was placed between the ultrasound exposure source and the animals under study [21]. Studies have previously

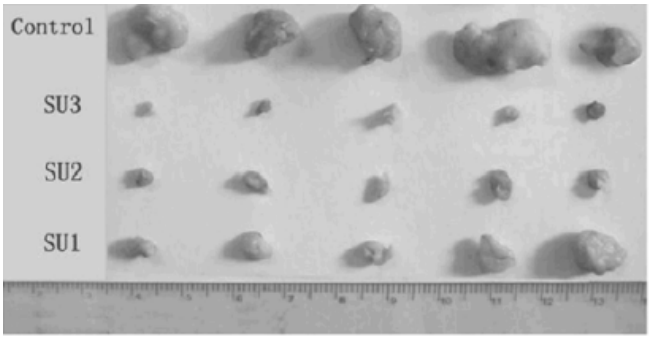


Fig. (3). Photograph of mouse S-180 tumours peeled off 15 days after treatment from each group of mice showing the effect of increasing the intensity of ultrasound exposure. Top line – Control sample without ultrasound or Sonnelux 1 administration. Second line (SU3) - highest ultrasound power used at 1. 2W/cm2, Third (SU2) and Fourth (SU1) lines are decreasing intensity of ultrasound (0.6W/cm2, 0.3W/cm2).

supported propogation of ultrasound through bone structure [22], and this provides further support for the possibility that sufficient ultrasound activation can be achieved for tumour sites distant or within bone structure.

METHOD

Activated Cancer Therapy Protocol

Sonnelux Protocol

Sonnelux-1 is administered slowly over 2 to 5 hours sublingually to provide sustained low plasma concentration. Forty eight hours after sublingual administration the patient is exposed to a light bed containing 48 panels of LED's emitting a combination of visible and infra-red light at the frequencies 660nm and 940nm (+/- 30nm).

No photosensitivity from normal ambient light, artificial or natural has been noted but as a precaution patients are advised not to stay in direct sunlight for periods over half an hour for one week following Sonnelux-1 administration. Light bed exposure time varies with shorter exposure duration in cases with larger tumour load.

Ultrasound is then applied at 1W/cm^2 and a frequency of 1MHz at sites of known malignant disease, with time titrated on a case by case basis.

Light and ultrasound activation is repeated on three consecutive days, and the same process of Sonnelux-1 administration followed by light and ultrasound exposure is repeated after one week to complete a treatment cycle.

Ozone auto-haemotherapy is administered immediately before light bed exposure, aiming to increase P02 at the tumour site. Clinically, this has been observed to significantly increase the tumour cytotoxic effect of SPDT.

A course of oral dexamethasone is administered to patients dependent on tumour type, background physical status and total tumour volume. Alongside SPDT protocol, patients underwent supportive nutritional supplementation determined on a case by case basis.

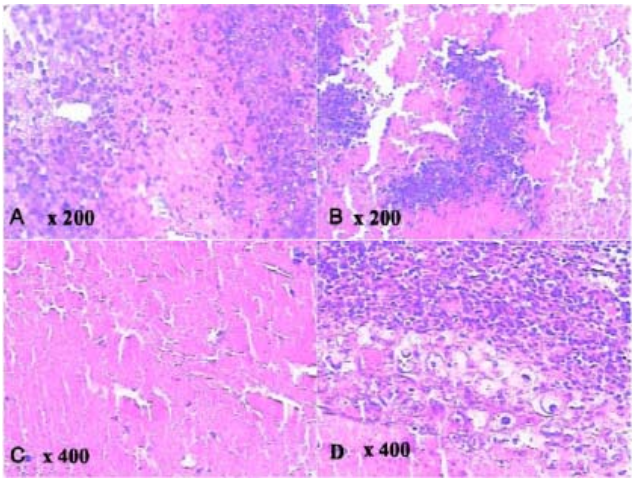


Fig. (4). Histological slices of the tumour in a group of mice sonnelux-1 plus ultrasound plus light exposure showing coagulated tumour cell necrosis, inflammatory changes and metamorphic tissue.

Slice taken 2 hours after treatment.

Slice taken 36 hours after treatment.

& D. Slices taken 15 days after treatment.

Data Collection

Details were collated of 115 consecutive patients who received SPDT, including hospital diagnosis, previous treatment, tumour staging and expected survival in months based on Oncologist opinion at initial consultation, where known. Patients were routinely followed up one month post treatment and subsequently at regular intervals. Clinical notes were reviewed and telephone contact was attempted to optimize data collation.

Results have been tabulated for comparison and a series of 3 cases are outlined in greater detail.

RESULTS

All patient data is anonymously displayed in the summary table (see Appendix **1**) according to primary diagnosis site. Patient data has only been graphically presented when a predicted median survival is known. Of those patients still alive, only those who have exceeded the predicted survival data are included in graphical representation.

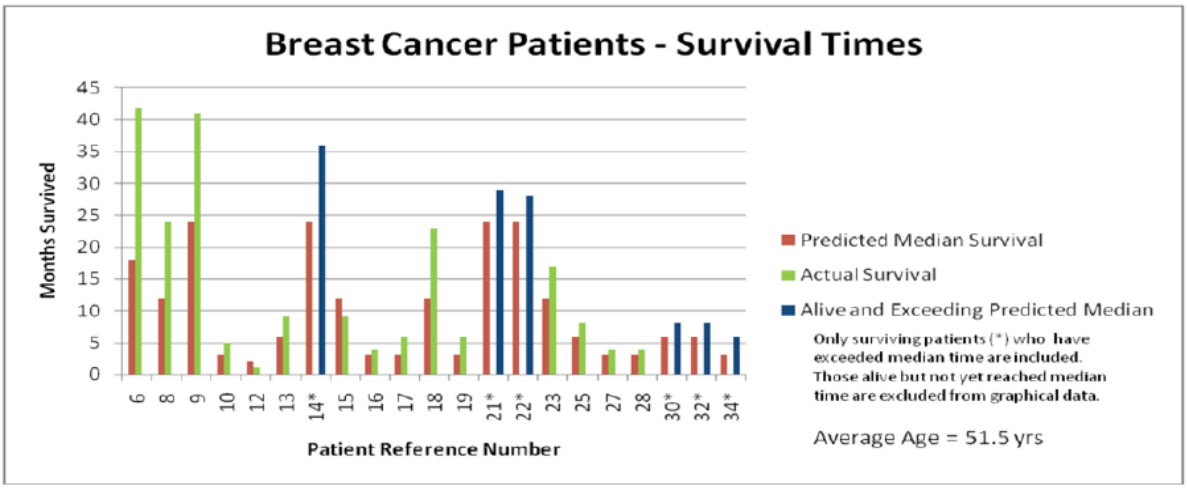
Many patients are alive at the time of writing; therefore survival benefit is unknown and has been given in months up to the time of writing.

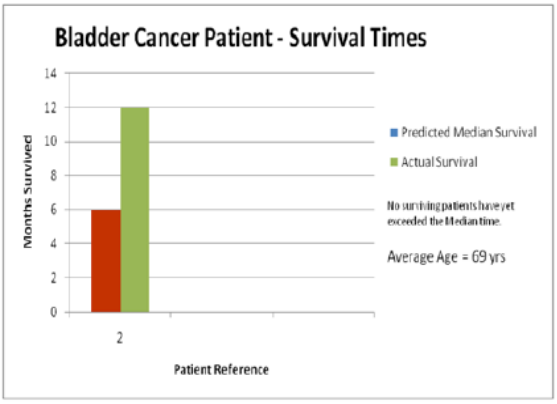
Case Reports

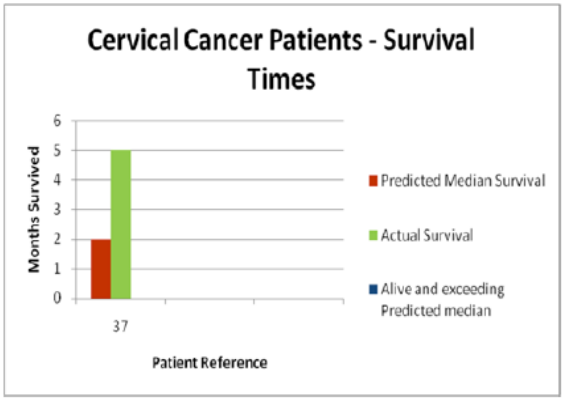
Case 1. Non Small-Cell Lung Cancer

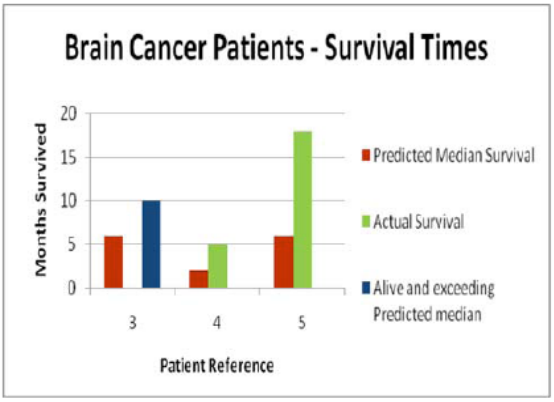
This 80 year old female patient presented in August 2005 with an inoperable non small-cell lung cancer in the left lung. She had refused palliative radiotherapy and at that time had been given a predicted median survival of 6 months. Sonnelux-1 protocol SPDT was completed in September 2005. Following treatment she developed an inter-scapula ache, but tolerated the treatment well. Until March 2007 she had stable disease, as determined by regular chest x-rays. In June 2007 she was demonstrated to have tumour progression

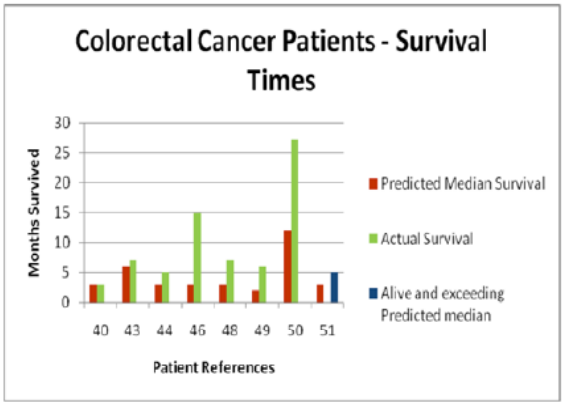
Graphical Data – Showing Predicted Median Survival and Actual Survival Times by Primary Diagnosis Site

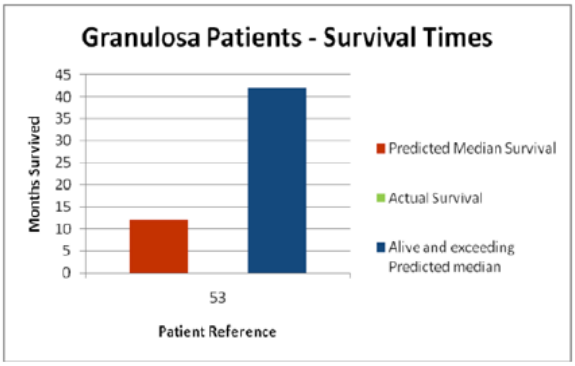


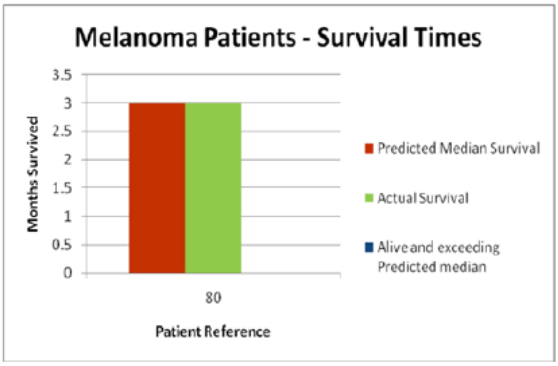












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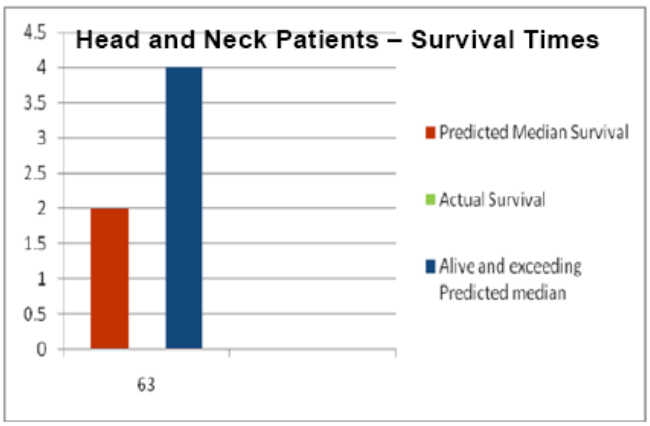
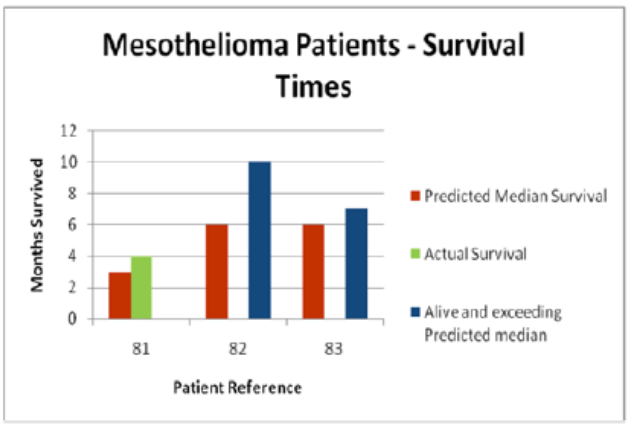
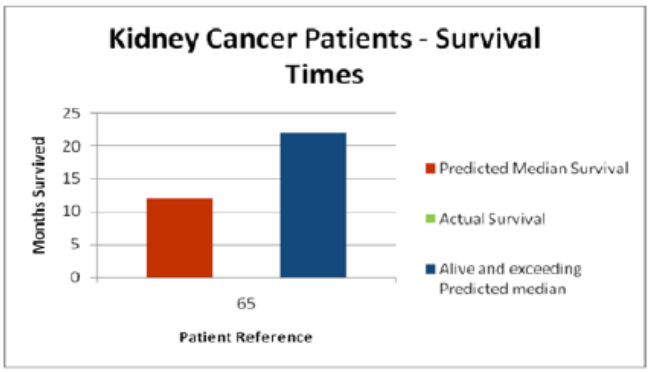
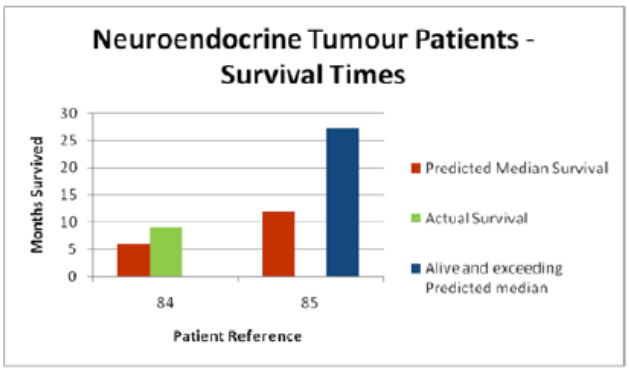
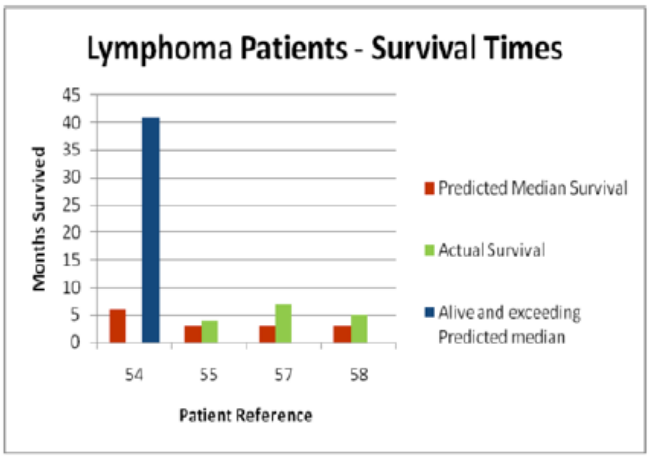
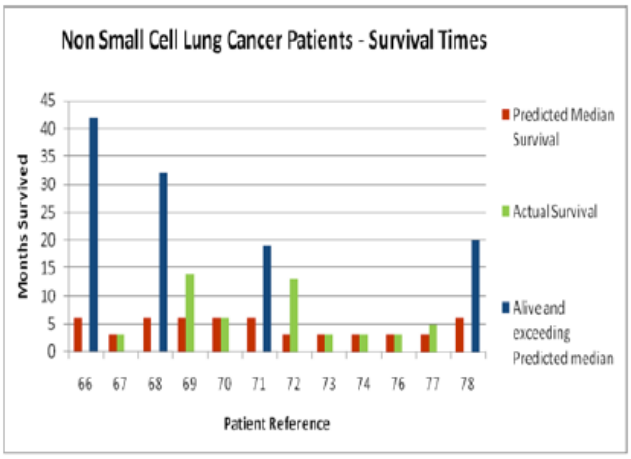

Head and Neck Patients – Survival Times
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
63
Predicted Median Survival
Actual Survival
Alive and exceeding Predicted median

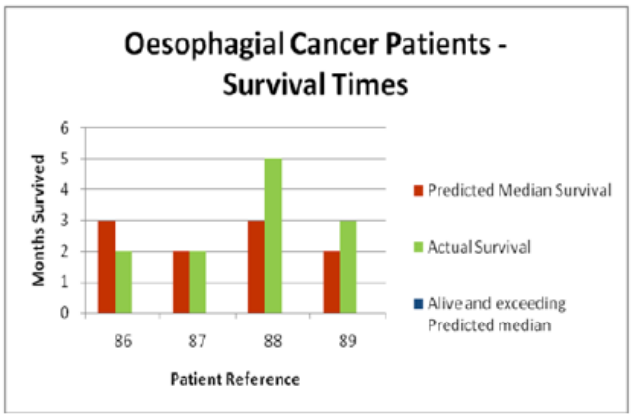

Mesothelioma Patients - Survival Times
Months Survived
12
10
8
6
4
2
0
81
82
83
Patient Reference
Predicted Median Survival
Actual Survival
Alive and exceeding Predicted median

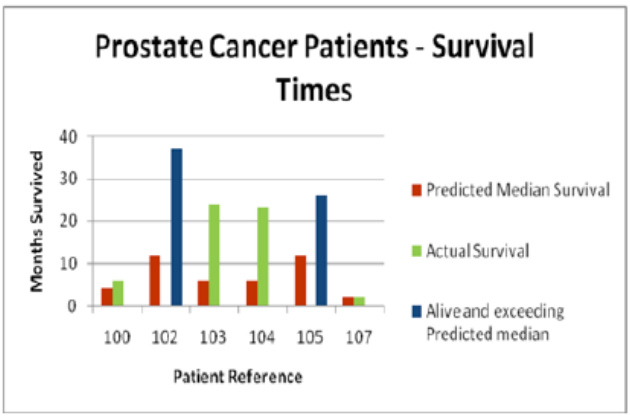

Kidney Cancer Patients - Survival Times
Months Survived
25
20
15
10
5
0
65
Patient Reference
Predicted Median Survival
Actual Survival
Alive and exceeding Predicted median


Neuroendocrine Tumour Patients - Survival Times
Months Survived
30
25
20
15
10
5
0
84
85
Patient Reference
Predicted Median Survival
Actual Survival
Alive and exceeding Predicted median

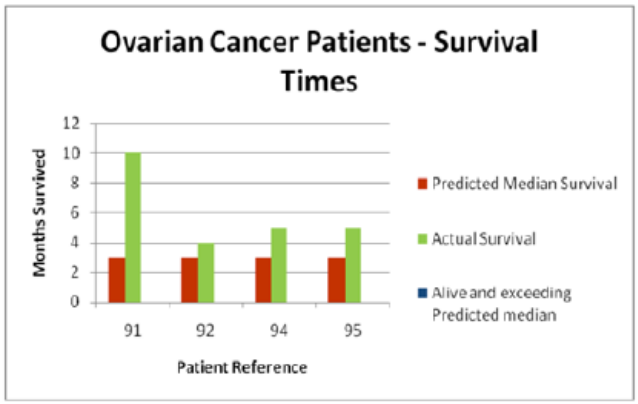

Lymphoma Patients - Survival Times
Months Survived
45
40
35
30
25
20
15
10
5
0
54
55
57
58
Patient Reference
Predicted Median Survival
Actual Survival
Alive and exceeding Predicted median

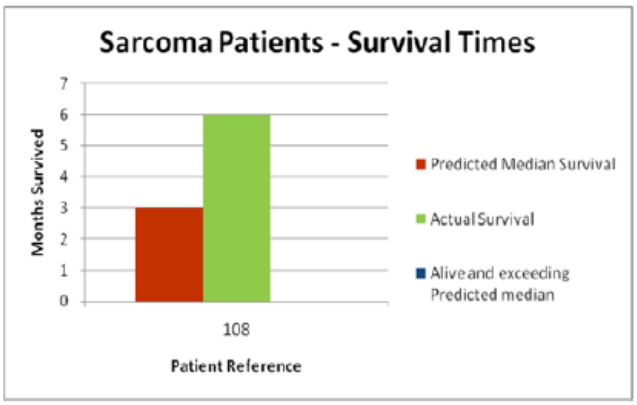

Non Small Cell Lung Cancer Patients - Survival Times
Months Survived
45
40
35
30
25
20
15
10
5
0
66
67
68
69
70
71
72
73
74
76
77
78
Patient Reference
Predicted Median Survival
Actual Survival
Alive and exceeding Predicted median

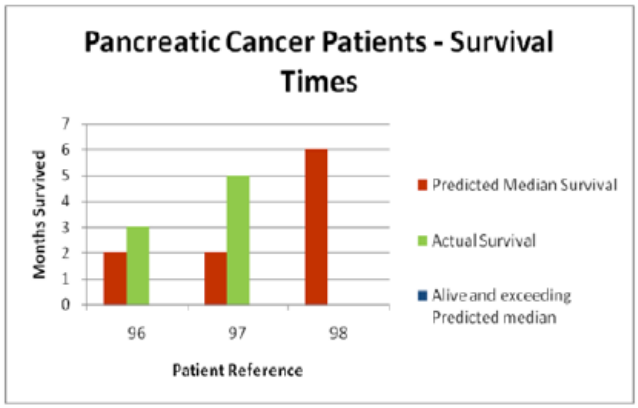

Oesophagial Cancer Patients - Survival Times
Months Survived
6
5
4
3
2
1
0
86
87
88
89
Patient Reference
Predicted Median Survival
Actual Survival
Alive and exceeding Predicted median

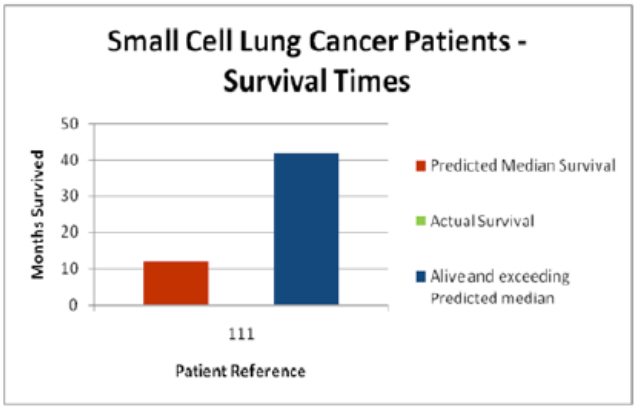

Prostate Cancer Patients - Survival Times
Months Survived
40
30
20
10
0
100
102
103
104
105
107
Patient Reference
Predicted Median Survival
Actual Survival
Alive and exceeding Predicted median

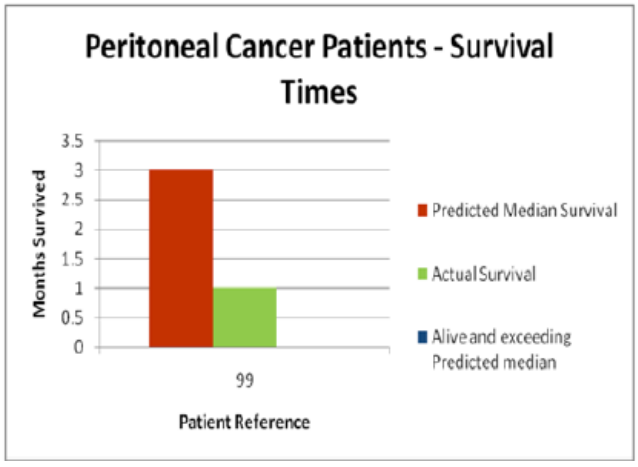
(Graph Contd….)

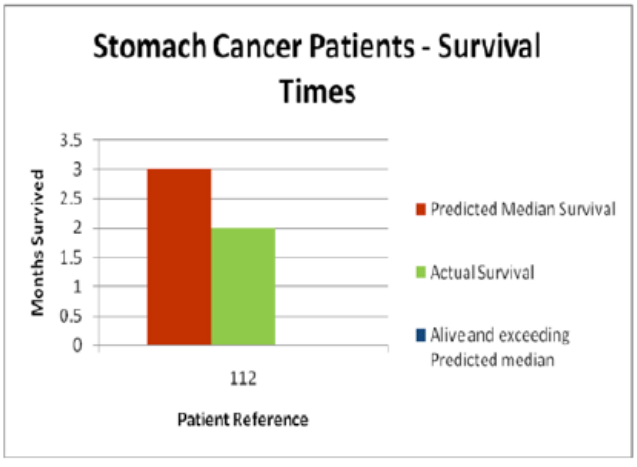

Ovarian Cancer Patients - Survival Times
Months Survived
12
10
8
6
4
2
0
91
92
94
95
Patient Reference
Predicted Median Survival
Actual Survival
Alive and exceeding Predicted median

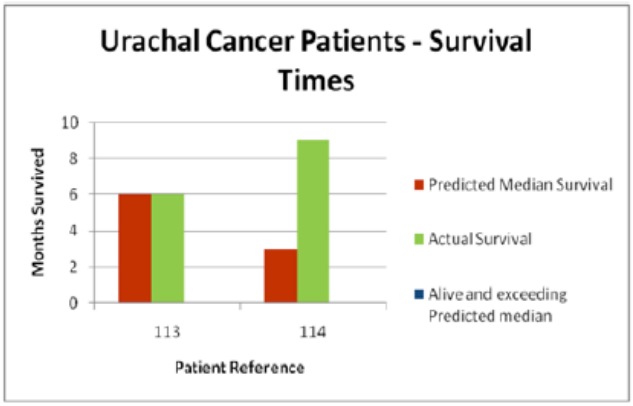

Sarcoma Patients - Survival Times
Months Survived
7
6
5
4
3
2
1
0
108
Patient Reference
Predicted Median Survival
Actual Survival
Alive and exceeding Predicted median

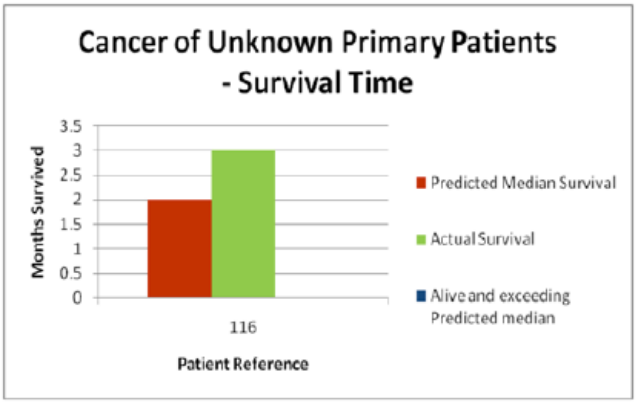

Pancreatic Cancer Patients - Survival Times
Months Survived
7
6
5
4
3
2
1
0
96
97
98
Patient Reference
Predicted Median Survival
Actual Survival
Alive and exceeding Predicted median


Small Cell Lung Cancer Patients - Survival Times
Months Survived
50
40
30
20
10
0
111
Patient Reference
Predicted Median Survival
Actual Survival
Alive and exceeding Predicted median


Peritoneal Cancer Patients - Survival Times
Months Survived
3.5
3
2.5
2
1.5
1
0.5
0
99
Patient Reference
Predicted Median Survival
Actual Survival
Alive and exceeding Predicted median


Stomach Cancer Patients - Survival Times
Months Survived
3.5
3
2.5
2
1.5
1
0.5
0
112
Patient Reference
Predicted Median Survival
Actual Survival
Alive and exceeding Predicted median


Urachal Cancer Patients - Survival Times
Months Survived
10
8
6
4
2
0
113
114
Patient Reference
Predicted Median Survival
Actual Survival
Alive and exceeding Predicted median


Cancer of Unknown Primary Patients - Survival Time
Months Survived
3.5
3
2.5
2
1.5
1
0.5
0
116
Patient Reference
Predicted Median Survival
Actual Survival
Alive and exceeding Predicted median

Appendix 1

Patient Number	Diagnosis - Primary Site	Description	Previous Treatment	Date of SPDT and First Consultation	Age at SPDT	Gender	2nd SPDT Course	Dexamethasone Course	Predicted Median Survival (Months)	Actual Survival (Months)	Survival Benefit (Months)	Doubled Predicted Survival	Comment
1	Bladder	TCC	Refused S	Dec' 08	50	M	N	N	Not known	Alive (3+)	Alive (?)	Not known	hydronephrosis resolved post SPDT
2 B	ladder	TCC, local recurrence, scapula met	S F	eb' 08	69	F	N	Y	6	12	6	Yes	passed necrotic tissue in urine
3 B	rain	Ependymoma, recent scan - reduced size, remains well	S R Refused C	Apr' 08	50	F	8-Dec	Y	6	Alive (10+)	Alive (4+)	No but alive	symptoms improved post SPDT, reduced tumour size on CT
4 B	rain	GBM	S	First seen Sep' 05 SPDT Nov' 08	56 M		N	Y	2	5	3	Yes	
5 B	rain	GBM	R	First seen Mar' 05 SPDT 8 2005	66 M		N	N	6	18	12	Yes	
6	Breast	ER +, bony mets	S C R, Ref tamoxifen	First seen Jul' 05 SPDT Aug' 05	41 F		N	N	18	42	24	Yes	
7 B	reast	Right intra-ductal ca, ER -ve, Her2 -ve,	S, Ref R Ref C	First seen Jul' 05 SPDT Aug' 05	41 F		N	N	Not Known	Alive (42+)	Alive (?)	Not known	neoadjuvant SPDT – necrotic tissue only at lumpectomy, recurrence free
8 B	reast	Grade 3 left side, ER-ve, Her2-ve, recurrent right sided ca	S, C	Sep' 05	67	F	N	N	12	24 +	lost to FU at 24/12 (12+)	Yes	
9	Breast	multiple bony mets	S C R	Aug' 05	52	F	N	N	24	41	17	No	
10	Breast	widespread mets	S C R	Oct' 05	49	F	N	N	3	5	2	No	
11 B	reast	widespread mets and local chest wall spread	S C R	First seen Jun' 05 SPDT Oct' 05	51 F		N	N	Not known	Lost to FU	Lost to FU	Not known	
12	Breast	bone mets	S C R	Nov' 05	55	F	N	N	2	1	-1	No	
13	Breast	bone and lung mets	S C R	First seen Dec' 05 SPDT Jan' 06	56 F		N	N	6	9	3	No	
14	Breast	bone mets	S C R	First seen Mar' 06 SPDT Apr' 06	53 F		N	N	24	Alive (35+)	Alive (11+)	No but alive	stable disease
15 B	reast	bone mets, pain settled post PDT, subsequent brain mets	S C R	First seen Mar' 06 SPDT Apr' 06	51 F		N	y	12	9	-3	No	

(Appendix Contd….)

Patient Number	Diagnosis - Primary Site	Description	Previous Treatment	Date of SPDT and First Consultation	Age at SPDT	Gender	2nd SPDT Course	Dexamethasone Course	Predicted Median Survival (Months)	Actual Survival (Months)	Survival Benefit (Months)	Doubled Predicted Survival	Comment
16 B	reast	widespread breast ca with bone and liver mets	S C R	First seen Oct' 05 SPDT 1 2006	30 F		N	N	3	4+ (lost to FU)	1+ (lost to FU)	No	
17 B	reast	bone liver brain and skin mets	S C R	First seen Jun' 06 SPDT Jul' 06	67 F		N	Y	3	6	3	Yes	
18 B	reast	left sided, Ref CT scan	No S C or R	Sep' 06	50	F	N	Y	12	23	11	No	initial inflammatory swelling around breast mass which settled over 3 months
19 B	reast	bone and liver mets	S C R	Sep' 06	67	F	N	Y	3	6	3	Yes	3 months pain in bony mets post SPDT, settled
20 B	reast	recurrence at scar site	S C R	Sep' 06	41	F	N	N	?	2+ lost to FU	lost to FU	Yes	post SPDT the recurrence reduced in size by over 50%
21	Breast	bone mets	S C R	First seen Oct' 06 SPDT Nov' 06	53 F		N	Y	24	Alive (29+)	Alive (4+)	No but alive	further scans shown no bony metastasis progression post SPDT
22	Breast	bone mets	S C R	Nov' 06	51	F	N	Y	24	Alive (28+)	Alive (4+)	No but alive	increased pain post SPDT
23 B	reast	skin mets and single bone met	S C R	First seen Jan' 07 SPDT Mar' 07	47	F	N	Y	12 17		5 N	o	
24 B	reast	node negative, ER -ve,, Her2 +ve	S	First seen Feb' 07 SPDT April' 07	51 F		N	N	Not known	Alive (12+)	Alive	Not known	
25	Breast	liver and bone mets	S C R	May' 07	38	F	N	Y	6	8	2	No	
26 B	reast	extensive local recurrence, ER and Her2 +	S C R	Aug' 07	67	F	Dec-07 and Jun-08	Y 24		Alive (18 +)	Alive	No but alive	3 x SPDT all with significant inflammatory response for 2 months post treatment
27 B	reast	brain and lungs mets	S C R	Oct' 07	57	F	N	Y	3	4	1	No	
28	Breast	bone and lung mets	S C R	Nov' 07	47	F	N	Y	3	4	1	No	bone pain and cough significantly improved post SPDT
29	Breast	grade 3, ER +	Ref C Ref R, Ukraine, S	First seen Apr' 05 SPDT Feb' 08	53 F		N	Y	Not known	Alive (12+)	Alive(?)	Not known	refused conventional neoadjuvant rx for recurrence, opted for neoadjuvant SPDT, excision biopsy of local recurrence showed necrotic cells only
30 B	reast	liver mets, ER -ve Her2 +	Herceptin S Ref C Ref R	Jul' 08	47	F	N	y	6	Alive (7+)	Alive	No but alive	liver metastasis reduced on scan but also on taxol chemo

(Appendix Contd….)

Patient Number	Diagnosis - Primary Site	Description	Previous Treatment	Date of SPDT and First Consultation	Age at SPDT	Gender	2nd SPDT Course	Dexamethasone Course	Predicted Median Survival (Months)	Actual Survival (Months)	Survival Benefit (Months)	Doubled Predicted Survival	Comment
31 B	reast	recurrence after 12 years	S C R	First seen Jun' 08 SPDT Jul' 08	46 F		N	N	Not known	lost to FU	lost to fu	Not known	
32	Breast	bone and lung mets	S C R	Jun' 08	60	F	N	Y	6	Alive (8+)	Alive (2+)	No but alive	
33 B	reast	liver and local LN secondaries ER -, Her2 +	S C R Her	First seen May' 08 SPDT Jul' 08	47 F		N	Y	12	Alive (10+)	Alive (?)	No but alive	stable disease on imaging
34 B	reast	liver, lymph node, bone , lung mets	S C R	Sep' 08	55	F	N	Y	3	Alive (6+)	Alive (3+)	Yes	
35 B	reast	right, node neg, ER and Her2 -ve	Ref C Ref R	First seen Nov' 08 SPDT Dec' 8	44 F		N	N	Not known	Alive (3+)	Alive (?)	Not known	
36 B	reast	bone mets, contra-lateral recurrence, met around optic nerve, pleural effusion	S R	Dec' 08	62	F	N	Y	6	Alive (3+)	Alive (?)	No but alive	pain, visual disturbance and wheeze significantly eased post SPDT
37 C	ervical	recurrence, stent right ureter, oe-dema right leg	C	First seen Jan' 07 SPDT Mar' 07	51 F		N	Y	2	5	2	Yes	swelling increased right leg post SPDT
38 C	ervical	recurrence , pelvic spread	C R for recurrence	First seen Feb' 08 SPDT Mar' 08	51 F		N	Y	Not known	Alive (11+)	Alive	Not known	
39 C	ervical	pelvic spread and local node	Ref further C	Nov' 08	50	F	N	Y	6	Alive (4+)	Alive	No but alive	initial swelling in inguinal glands and increased pelvic pain, settled
40 C	olorectal	liver mets, ER -ve Her2 +	S N	ov' 05	64	F	N	N	3	3	0	No	
41 C	olorectal	liver mets, ER -ve Her2 +	Ref C S	Oct' 05	56	M	N	N	6	lost to FU	lost to FU	Not known	
42 C	olorectal	hemicolectomy, lung mets - left upper lobectomy	S, Ref C	First seen Oct' 05 SPDT Jan' 06	66 F		N	N	Not known	Alive, (40+) disease free	Alive (?)	Not known	well and disease free
43	Colorectal	lung and liver mets	S C	Jan' 06	64	M	N	N	6	7	1	No	
44	Colorectal	liver mets	S C	Oct' 05	29	M	N	N	3	5	2	No	
45	Colorectal	liver mets	S C	Jul' 06	65	M	N	N	6	lost to FU	lost to FU	No	
46	Colorectal	liver mets	S C	Jan' 07	48	F	N	Y	3	15	12	Yes	
47 C	olorectal	SCC anus, liver met	R C,S - partial hepatectomy	Jan' 07	54	F	N	N	Not known	Alive (17+)	Alive	Not known	neoadjuvant SPDT - liver lesion - necrotic cells only

(Appendix Contd….)

Patient Number	Diagnosis - Primary Site	Description	Previous Treatment	Date of SPDT and First Consultation	Age at SPDT	Gender	2nd SPDT Course	Dexamethasone Course	Predicted Median Survival (Months)	Actual Survival (Months)	Survival Benefit (Months)	Doubled Predicted Survival	Comment
48 C	olorectal	peritoneal mets	S C	Nov' 07	56	M	N	Y	3	7	4	Yes	initial abdo pains post SPDT
49 C	olorectal	liver and lung mets	S C	Apr' 08	48	F	N	Y	2	6	4	Yes	
50 C	olorectal	rectal ca	ref S	Sep' 06	74	M	7-Jun	N 12		lost to fu (27+)	lost to fu 15+	Yes	
51 C	olorectal	lung and liver mets	ref C, S	Oct' 08	70	F	N	Y	3	Alive (5+)	Alive (2+)	No	
52 C	olorectal	lung mets, right ureter stent	S C	Jan' 09	64	M	N	Y	Not known	Alive (1+)	Alive (?)	Not known	back pain from pelvic mass and right sided peripheral oedema - started to ease 10 days post SPDT
53	Granulosa Cell	mets around porta-hepatis	A	ug' 05	63	F	N	N	12	Alive (42+)	Alive (30+)	Yes	well, some residual tumour
54 L	ymphoma	recurrent NHL	C	Jul' 05	60	F	N	N	6	Alive (41+)	Alive Ye	s	resistant to 2nd line chemo-in full remmission post SPDT
55 L	ymphoma	HL	C	Sep' 05	69	M	N	N	3 4		1	No c	hemo resistant
56 L	ymphoma	recurrent NHL, large gland left neck	C M	ay' 06	55	F	N	N	Not Known	lost to FU(2+)	lost to FU	Not known	neck node 25% of original size 2/12 post rx
57 L	ymphoma	recurrent NHL	C	Jun' 07	59	F	N	N	3	7	4	Yes	chemo resistant, significant reduction in tumour size one month post SPDT
58 L	ymphoma	recurrent NHL	C	Feb' 08	64	M	N	N	3	5	2	No	chemo resistant
60 L	ymphoma	recurrent NHL	C	Aug' 08	55	F	N	N	Not known	Alive (6+)	Alive (?)	Not known	palpable enlarged lymph nodes reduced in size by 40%
61	Head & Neck	SCC tongue, lung mets		Apr' 06	58	M	N	N	12	lost to FU	lost to FU	Not known	
62	Head & Neck	recurrent mouth SCC	S, Ref further S & C	Oct' 08	60	M		Refused	6	Alive (4+)	Alive (?)	No but alive	refused dexamethasone and developed swallowing difficulty, required PEG insertion, swallowing difficulty now starting to resolve
63	Head & Neck	SCC larynx	S Ref R, already had R for previous lymphoma so refused further R	Oct' 06	58	F	8-Nov	Y	2 (at recurrence in Nov-08)	Alive (3+)	Alive	No but alive	neoadjuvant SPDT 06. Recurrence 08 distant spread, further SPDT performed. Developed trigeminal neuralgia post SPDT
64	Head & Neck	Adenocarcinoma pallet, large LN right neck	J	an' 09	58	F	N	Y	Not known	Alive (1+)	Alive (?)	Not known	tumour mass reduced by 50%, now operable, aim for post op SPDT

(Appendix Contd….)

Patient Number	Diagnosis - Primary Site	Description	Previous Treatment	Date of SPDT and First Consultation	Age at SPDT	Gender	2nd SPDT Course	Dexamethasone Course	Predicted Median Survival (Months)	Actual Survival (Months)	Survival Benefit (Months)	Doubled Predicted Survival	Comment
65 K	idney	lung and pancreas(?) mets	monoclonal antibody post SPDT	Apr' 07	56	M	N	12		Alive (22+)	Alive (10+)	No but alive	scan showed tumour progression 4/12 post SPDT
66 L	ung-NSCLC	inoperable r	ef R	First seen Aug' 05 SPDT Sep' 05	80 F		7-Jun	N	6	Alive (42+)	Alive (36+)	Yes	initial inter-scapular ache initially, remains well, disease stable on X Ray
67 L	ung-NSCLC	liver and lung mets	D	ec' 05	37	F	N	N	3	3	0	No	
68 L	ung-NSCLC	left upper lobe		Jun' 06	79	F	N	N	6	Alive (32+)	Alive (26+)	Yes	well with stable disease
69 L	ung-NSCLC	Right lower lobe	A	ug' 06	61	F	N	Y	6	14	8	Yes	
70 L	ung-NSCLC	Left lower lobe		Jul' 06	49	M		Y	6	6	0	No	
71 L	ung-NSCLC	right adrenal met	Ref C	Jul' 07	56	F	7-Oct	Y	6	Alive (19+)	Alive (13+)	Yes	cough resolved post SPDT, further SPDT when became breathless
72 L	ung-NSCLC	brain mets	R, ref R lung	Nov' 07	69	M	N	Y	3	13	10	Yes	
73 L	ung-NSCLC	muscle met		Apr' 07	79	F	N	Y	3	lost fu (3+)	lost to fu (0+)	Not known	
74 L	ung-NSCLC	right lower lobe, brain mets	M	ar' 08	67	M	N	Y	3	3	0	No	dry persistent cough improved post SPDT
75 L	ung-NSCLC	left lung	C	Nov' 08	70	F	N	Y	4	Alive (3+)	Alive (?)	No but alive	cough and SOB improved post SPDT
76 L	ung-NSCLC	brain and bone mets	O	ct' 08	62	M	N	Y	3	3	0	No	
77 L	ung-NSCLC	right lung		Mar' 08	53	F	N	Y	3	5	2	No	
78 L	ung-NSCLC	bone and adrenal mets, previous adeno-ca right lung	C R	First seen Jun' 07 SPDT Jul' 07	79 F		N	Y	6	Alive (20+)	Alive (14+)	Yes	bone mets pain resolved 1/12 post SPDT
79 L	eukaemia	Relapsed Acute Myeloid Leukaemia	C N	ov' 08	50	F	N	Y	Not known	Alive (3+)	Alive (?)	Not known	chemoresistant, ? Ineffective, no change in pancytopenia
80 M	elanoma	brain met and skin mets	M	ar' 06	60	M	N	N	3	3	0	No	
81 M	esothelioma	right sided	C	Aug' 07	71	M	N	Y	3	4	1	No	
82 M	esothelioma	right sided		First seen Apr' 08 SPDT May' 08	62 M		N	Y	6	Alive (10+)	Alive (4+)	No but alive	
83 M	esothelioma	right sided	Ref R C	Jul' 08	67	M	N	Y	6	Alive (7+)	Alive (1+)	No but alive	

(Appendix Contd….)

Patient Number	Diagnosis - Primary Site	Description	Previous Treatment	Date of SPDT and First Consultation	Age at SPDT	Gender	2nd SPDT Course	Dexamethasone Course	Predicted Median Survival (Months)	Actual Survival (Months)	Survival Benefit (Months)	Doubled Predicted Survival	Comment
84 N	ET	bronchial carcinoid, liver mets	C R	Dec' 05	39	F	N	N	6	9	3	No	
85	NET	bone mets	C	Nov' 06	50	F	N	Y	12	27	Alive (15+)	Yes	pain in bone mets for one week
86 O	esophagus			Apr' 06	56	M	N	Y	3	2	-1	No	
87 O	esophagus	bone and brain mets	C S R	First seen Apr' 06 SPDT May' 06	47 M		N	Y	2	3	1	No	
88	Oesophagus	stent in situ	Ref C Ref R	First seen Sep' 07 SPDT Nov' 07	64 M		N	Y	3	lost to fu (5+)	lost to fu (2+)	Not known	swallow and appetite improved post SPDT
89	Oesophagus	liver mets		Jan' 08	61	M	N	Y	2	3	1	No	
90 O	varian	stage 1c	Refused all conventional treatment	Jul' 05	62	F	N	N	Not known	Alive (43+)	Alive	Not known	no conventional treatment, tumour free post SPDT
91 O	varian	recurrent	C	Aug' 05	62	F	N	N	3	10	7	Yes	
92 O	varian	recurrent	C	Fisrt seen Nov' 05 SPDT Dec' 05	50 F		N	N	3	4	1	No	
93	Ovarian	recurrent	Ref C	Feb' 06	52	F	N	N	6	lost to fu	lost to fu	Not known	
94 O	varian	recurrent	C	Oct' 06	63	F	N	Y	3	5	2	No	CT post SPDT - reduced size of pelvic mass, large piece of necrotic tissue lost PV,initial abdominal pain
95 O	varian	recurrent	C	May' 07	43	F	N	N	3	5	2	No	
96 P	ancreas	recurrent, lung and throat mets		Fisrt seen Apr' 06 SPDT May' 06	70 M		N	Y	2	3	1	No	
97 P	ancreas			First seen Sep' 07 SPDT Dec' 07	61 M		N	Y	2	5	3	Yes	
98 P	ancreas	local, hx myelodysplasia	D	ec' 07	77	F	N	Refused	6	0	minus 6 (died 2y to CVA)	No	Cerebral Infarct 1/52 post SPDT
99	Peritoneal	C		Jan' 06	57 M		N	N	3	1	-2	No	chemoresistant
100 P	rostate	pelvic and bone mets	J	un' 05	72	M	N	N	4	6	2	No	pain initially worse then resolved over two months
101	Prostate	recurrent, LN	S, Zoladex	First seen Sep' 05 SPDT Oct' 05	71 M		N	N	Not known	36	Not known	Not known	died of CVA

(Appendix Contd….)

Patient Number	Diagnosis - Primary Site	Description	Previous Treatment	Date of SPDT and First Consultation	Age at SPDT	Gender	2nd SPDT Course	Dexamethasone Course	Predicted Median Survival (Months)	Actual Survival (Months)	Survival Benefit (Months)	Doubled Predicted Survival	Comment
102	Prostate	gleason 7	S R	Jan' 06	59	M	N	N	12	Alive (37+)	Alive (25+)	Yes	having radiotherapy for recent rising PSA
103 P	rostate	bone mets	Zoladex	First seen Jul 06' SPDT Aug' 6	55 M		N	N	6	24	18	Yes	initial bony pain then settled
104 P	rostate	recurrent prostate and pancreatic ca	Zoladex	First seen Sep' 06 SPDT Nov' 06	66 M		N	N	6	23	17	Yes	
105 P	rostate	bone mets	Brachy-therapy	Dec' 06	59	M	N	N	12	Alive (26+)	Alive (14+)	Yes	bone pain improved post SPDT
106 P	rostate	local extension and LN involvement	Zoladex	First seen Jul' 06 SPDT Aug' 6	66 M		6-Dec	N	Not known	Alive (31+)	Alive (?)	Yes	
107 P	rostate	Liver mets and LN involvement	Zoladex	Jan' 08	57	M	N	Y	2	2	0	No	one week SPDT cycle only
108 S	arcoma	leiomyosarcoma, heart, pancreas and lung mets	S	First seen Sep' 05 SPDT Nov' 05	42 F		N	Y	3	6	3	Yes	
109 S	arcoma	synovial, lung and poericardial mets	S	First seen Sep' 08 SPDT Nov' 08	50 F		N	Y	6	Alive (5+)	Alive No		
110 S	arcoma	recurrent fibrosar-coma left chest	S A	pr' 06	34	F	N	N	Not known	lost to fu	lost to fu	Not known	
111	Lung - Small cell lung cancer	C		Aug' 05	61	F	N	N	12	Alive (42+)	Alive (30+)	Yes	Subsequent scan showed 80% reduction in tumour size, remains well
112 S	tomach	recurrent	C	Sep' 05 45		F	N	N	3	2	-1	No	
113 U	rachal	recurrent	C	Nov' 06	39	F	N	N	6	6	0	No	
114 U	rachal	recurrent	nephrostomy	Mar' 08	63	M	N	Y	3	9	6	Yes	tumour mass decresed significantly 2/12 post SPDT
115	Ca of unkown primary	pelvic mass	S	Jan' 08	40	F	N	Y	Not known	Alive Aliv e		Not known	tumour reduced in size and nodular appearance post SPDT, progression next scan
116	Ca of unkown primary	abdo LN's +/ abdo mets	C	First seen Mar' 08 SPDT Apr' 08	67 F		N	N	2	3	1	No	

Key: S = surgery, C= chemotherapy, R= radiotherapy, Ref= patient declined specified treatment, mets= metastasis, ? = data not known, ER= oestrogen receptor, FU= follow up, GBM= gliobastoma multiforme, TCC= transitional cell carcinoma, pt number 59 allocated blank in error therefore not included in data table, HL= Hodgkin's Lymphoma, NHL= non-Hodgkin's Lymphoma, NSCLC= non small cell lung cancer.

and underwent a second course of Sonnelux-1 protocol at that time. She tolerated the second course well and at the time of writing (February 2009) she still has stable disease on chest x-rays and is symptom free, with a good quality of life.

Case 2. Brain Tumour – Ependymoma

This 50 year old female patient presented in April 2008, with a massive ependymoma first diagnosed in April 2003. At first consultation her clinical state was poor, with a predicted median survival time of 6 months. She had previously undergone surgical de-bulking and whole brain radiotherapy. She had refused management with Temozolamide. Sonnelux-1 protocol was performed in April 2008. Dexamethasone was prescribed for the treatment course (2mg twice a day). A month after treatment she felt well enough to go on a 2 month holiday abroad. She has remained relatively symptom free. A further course of sonnelux-1 protocol was performed in October 2008. Repeat CT scans in December 2008 showed that the tumour had decreased in size.

Case 3. Non-Hodgkin's Lymphoma

This 60 year old female patient presented following a recurrence of non-Hodgkin's lymphoma which was resistant to second line chemotherapy. Sonnelux-1 protocol SPDT was completed in July 2005. At the time of writing, she is in full remission and has no recurrence of her tumour, with no other active treatment having been carried out.

DISCUSSION

Activated Cancer Therapy using Sonnelux protocol shows significant promise over a 4 year period as a safe and well tolerated non-invasive treatment even in advanced metastatic cancer. Extension in median survival times have been reported in a number of patients with a variety of cancer diagnoses. There are several patients still alive with reduced tumour mass and stable disease both clinically and on imaging. No adverse events were noted following administration of Sonnelux-1.

Second and subsequent courses of ACT may have further benefit in reducing tumour mass and inhibiting tumour cell growth without the total dose limitations of radiotherapy. Initial observation suggests that for patients with extensive tumour mass it is better tolerated to undertake ACT using shorter cycles of light and ultrasound activation with dexamethsone cover. This approach controls the acute inflammatory response demonstrated on excision biopsy in previous animal studies [21] and those seen in this case series with initial inflammatory changes at tumour sites.

While the inflammatory phase must be controlled, preclinical studies suggest that successful treatment outcome following PDT is critically dependent on the contribution from the host's acute-phase inflammatory response [23].

It is suggested that unlike immunologically silent genotoxic damage produced by radiotherapy and chemotherapy, photo-oxidative cytotoxic lesions generated by PDT are extra-nuclear and result in a rapid cell death that alerts the host's innate immune system. [24]. Neutrophil mobilisation and innate immune cell activation are responsible for the development of tumor antigen-specific adaptive immune cascades that contribute to the eradication of PDT-treated cancers. This is further supported by *in vitro* studies which established that tumour cells treated by PDT can be used for generating potent vaccines against cancers of the same origin [25].

Exacerbation of bony metastasis pain has been recorded, often followed by a reduction or resolution of pre-existing bony pains. It therefore appears that sufficient ultrasound activation of Sonnelux-1 can occur within and distal to bony structures to achieve tumour cell inhibition. This finding is supported by previous animal studies [21] and the improvement in symptoms and CT appearance of a patient with a large intracranial ependymoma.

There also appears to be a potential role for ACT in neoadjuvant cancer treatment, with necrotic tissue on excision biopsy at tumour sites occurring within this case series.

Tumour hypoxia has been found to be a characteristic feature in many solid tumours [26]. It has been demonstrated that tumor hypoxia, either pre-existing or as a result of oxygen depletion during photodynamic therapy can significantly reduce the effectiveness of PDT-induced cell killing [27]. This study reported that when PDT is combined with hyperoxygenation, the hypoxic condition could be improved and the cell killing rate at various time points after ACT could be significantly enhanced [27].

Previously it has been shown in arteriopathic patients that ozone autohemotherapy has a therapeutic potential by increasing oxygen delivery in hypoxic tissues [28]. Clinically, it appears that greater tumour response is seen with ACT following ozone autohaemotherapy. This may relate to an increase in PaO2 in the tumour microenvironment.

Unlike other cancer treatment modalities no bone marrow suppression has been noted following ACT. Patients underwent pre and post routine blood testing. Although not statistically assessed, haemoglobin, total white cell count and platelet count appear unchanged throughout the treatment and follow up period.

CONCLUSION

ACT (SPDT) warrants further investigation as a non-invasive, well-tolerated and clinically effective targeted cancer treatment capable of tumour cell necrosis at both superficial and deep malignant sites. There is increasing evidence supporting the mechanism of action of Activated Cancer Therapy using light and ultrasound and this case series reports on several patients with significant extension in median survival times with a variety of cancer diagnoses, showing reduced tumour mass and stable disease both clinically and on radiographic imaging.

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