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**The method of treating cancer**

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(54) Title: THE METHOD OF TREATING CANCER

(57) Abstract: Present invention relates to the method of treating cancer. According to present invention, a pharmaceutical composition made from "Mycobacterium w' ($M_w$) is found to be useful in the management of cancer. We have now found that the same therapeutic agent is useful in management of cancer. The use of Mycobacterium w containing formulations is associated with decrease in burden of cancer tissue, decreasing symptoms associated with cancer and improving quality of life. It also improves tolerance to other therapies.

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# THE METHOD OF TREATING CANCER

This invention relates to the method of treating cancer.

## BACKGROUND OF THE INVENTION

Cancer is believed to be caused by defective immune system. Many attempts have been made to improve immune system without success. Surprisingly it is found that Mycobacterium w containing compositions which are useful in improving immune status in patients with leprosy are also useful in management of cancer. They are found to be useful in decreasing burden of disease and reducing symptoms associated with cancer. More surprising was their synergy with conventional therapy inspite of fact that they work through entirely different mechanism. Still surprising was decrease in side effects of other therapy rather than increase in overall side effects inspite of use in same therapeutic amount alongwith increase in effect.

## Prior Art

Treatment of cancer is has traditionally been approached though chemotherapy, coupled with radiotherapy for primary elimination of leukemias, neoplasams and tumours. In contrast surgery has been used to remove solid tumours. Therapy involves both curative and palliative leading to cure and reduction of suffering of the patient. Immunotherapetic methods have also been found to be effective against a restrictive range of tumours of mesodermal origin suggesting that the immune system is capable of preventing or capable of delaying the growth of tumours in certain cases.

Traditionally BCG vaccine is used for boosting of immunity of individuals with cancer. This has not been well accepted as a mode of therapy due to inconclusive results. The only accepted method of BCG is to use it for bladder cancer by way of intravesicular therapy. The disadvantage associated with use of BCG is development of systemic and local tuberculosis caused by BCG. This is related to the fact that BCG contain live organism and they can be pathogenic to immunocompromised host.

US Patent 6,030,618 discloses an invention related to compositions, methods and kits for the prevention and treatment of primary and metastatic cancers and /or infectious diseases using heat shock/stress proteins (hsp) alone or in combination with each other and antigenic molecules to augment the immune responses to genotoxic and nongenotoxic factors, tumors, pathogens and infectious agents.

US Patent 5,767,156 provides a method of stimulating macrophage neutrophil and/or monocyte function in a subject. The method involves the administration of an effective amount of a free fatty acid having 18 – 24 carbon chain length with 2-6 double bonds and TNF or a TNF fragment or GMCSF or interferon gamma.

US Patent 6,080,725 is directed to vaccines comprising one or more bacterial, viral, or tumour-associated antigens; and one or more saponin-lipophile conjugate in which the lipophilic moiety such as a lipid, fatty acid, PEG, or terpene is covalently bonded to a non-acylated or deacylated triterpene saponin via a carboxyl group present on the 3-O-glucuronic acid of the triterpene saponin. The bacterial antigen in the vaccine are associated with a bacterial selected from diverse groups of bacteria including Mycobacterium tuberculosis.

US Patent 6,221,351 B1 relates generally to tumouricidal compositions and methods and more specifically to superantigens or enterotoxins derived from Staphlococcus aureus. Peptides homologus to the enterooxins including shock syndrome toxin, Srreptococcal pyrogenic exotoxins, mycoplasma and mycobacterial species, minor lymphocyte stimulating antigens, heat shock proteins, stress\peptides, mammary tumour virus peptides, homologous synthetic polypeptides, biochemically derivatised enterotoxins, genetically engineered enterotoxins and fusion proteins. This invention also relates to superantigens expressed on the surface of lipid droplets in adjuvant-vehicle formulations or expressed in biologic cell surfaces as a result of enterotoxin gene transfection and used to produce a tumourocidal response in tumour bearing hosts. It also relates to enterotoxins and related compounds administered intravenously, subcutaneously, as in adjuvant form, or used extracorporeally in free or bound form to stimulate immunocytes, which are subsequently infused into tumour bearing tissues.

US Patent 6,090,385 discloses a method of treating a cancer patient which comprises administering to said patent an anti-tumour effective amount of at least one of a water-soluble thermostable macromolecular antigen complex which is interspecific of microorganisms of the Mycobacteria, Nocardia, and Corynebacteria group and which exhibits after electrophoresis an innumo-electrophoretic precipitation pattern corresponding to that of the antigen complex 60 of the Mycobacterium bovis Clmette Guerin Bacillus strain, or immunogenic fragments of such a complex. It comprises an additional step of administering a therapeutic agent specific against the patient's cancer.

US Patent 6,056,964 suggests the delaying or preventing the growth oe spread of brest or bronchial neoplasm which comprises administering to a subject in need of the same, antigenic and/or immunoregulatory material whih comprises killed cells of Mycobacterium vaccae strain NCTC 11659 in an amount sufficient at least to delay or prevent the growth or spread of said neoplasm. This could be administered by intradermal injection.

US Patent 6,033,669 describes a method of stimulating the generation of cytotoxic T Cells (CTLs) in a patient, wherein the CTLs have the potential to destoy or attenuate cells presenting a characteristic disease-associated carbohydrate structure, which comprises administering to the patient an effective dose of a peptide/carbohydrate conjugate complex capable of generating cytotoxic T cell immunity against a carbohydrate structure, said conjugate structure comprising (i)a peptide component capable of binding an MHC class I molecule, and (ii) a carbohydrate component comprising of

immunogenic specificity of said disease-associated carbohydrate structure and being of a size that enables a T cell receptor to encompass an epitope of said disease-associated carbohydrate structure. This is claimed to be effective for treatment of melanoma, breast cancer, lung cancer or gastrointestinal cancer.

It has surprisingly been found that pharmaceutical compositions containing Mycobacterium W are effective in the treatment of a broad range of cancer indications. Pharmaceutical compositions as per present invention may contain extracts of Mycobacterium W alone or in combination of Mycobacterium W. As per another aspect of present invention pharmaceutical composition may contain other immunomodulator. It can be administered in various ways including intradermal, oral, intralesional etc. The present invention discloses such formulations and the method of their manufacture and use.
Mycobacterium W is a rapidly growing Mycobacterium which is not a pathogen.

Mycobacterium w is a non-pathogenic, cultivable, atypical mycobacterium, with biochemical properties and fast growth characteristics resembling those belonging to Runyons group IV class of Mycobacteria in its metabolic and growth properties but is not indentical to those strains currently listed in this group. It is therefore thought that ($M_W$) is an entirely new strain. The species identity of $M_W$ has been defined by polymerase chain reaction DNA sequence determination.

It has been found to share antigens with Mycobacterium leprae and Mycobacterium tuberculosis. It is found to provide prophylaxis against leprosy in humans by converting lepromin negative individuals to lepromin positivity. It is also found to provide prophylaxis against tuberculosis in animals. In leprosy it is also found to reduce duration of therapy for bacterial killing, clearance as well as clinical cure when used along with multi drug therapy.

**Summary of the Invention**

According to a first aspect of the invention there is provided a method of treating cancer comprising administration of a formulation which is prepared using mycobacterium w or a pharmaceutical composition obtained from mycobacterium w alone or in combination and also with or without adjuvants to a subject who has been suffering from cancer.

According to a second aspect of the invention there is provided the process for manufacturing a pharmaceutical composition useful for management of cancer comprises of incorporating cells of mycobacterium w along with pharmaceutically acceptable carrier and optionally a preservative in a single formation wherein cells of mycobacterium w are not alive.

According to a third aspect of the invention there is provided the process of manufacturing a pharmaceutical composition useful for management of cancer comprising the steps of incorporating disrupted cells of mycobacterium w along with pharmaceutically acceptable carrier and optionally a preservative.

According to a fourth aspect of the invention there is provided the process of manufacturing a pharmaceutical composition useful for management of cancer comprising the steps of incorporating solvent extraction of mycobacterium w along with pharmaceutically acceptable carrier and optionally a preservative.

According to a fifth aspect of the invention there is provided the process of manufacturing a pharmaceutical composition useful for management of cancer comprising of incorporating enzymatic extraction of mycobacterium w along with pharmaceutically acceptable carrier and optionally a preservative.

According to a sixth aspect of the invention there is provided the enzymes used for enzymatic extraction of cells of mycobacterium w is selected from lyticase and/or pronase.

According to a seventh aspect of the invention there is provided the use of mycobacterium w or constituents of mycobacterium w in the preparation of a pharmaceutical composition for use in treating or managing cancer.

According to another aspect of the invention there is provided the use of mycobacterium w or constituents of mycobacterium w or constituents of mycobacterium w in the preparation of a pharmaceutical composition for use in decreasing the burden of cancer tissue.

According to present invention, a pharmaceutical composition made from 'Mycobacterium w' ($M_w$) is found to be useful in the management of cancer. We have now found that the same therapeutic agent is useful in management of cancer. The use of mycobacterium w containing formulations is associated with decrease in burden of cancer tissue, decreasing symptoms associated with cancer and improving quality of life. It also improves tolerance to other therapies.

Therapeutic agent which may be used in the present invention resembles Mw a non-pathogenic, cultivable, atypical mycobacterium with biochemical properties and fast growth characteristics resembling those belonging to Runyons group IV class of Mycobacteria in its metabolic and growth properties but is not identical to those strains currently listed in this group. It is therefore thought that ($M_w$) is an entirely new strain.

The species identity of Mw has been defined by polymerase chain reaction DNA sequence determination and differentiated from thirty other species of mycobacteria. It however differs from those presently listed in this group in one respect or the other. By base sequence analysis of a polymorphic region of pattern analysis, it has been established that $M_w$ is a unique species distinct from many other known mycobacterial species examined which are: M. avium, M. intracellulare, M. scrofulaceum, M. kanasasii, M. gastri, M. gordonae, M. shimoidei, M. malmoense, M. haemophilum, M. terrae, M. nonchromogenicum, M. triviale, M. marinum, M. flavescens, M. simian, M. szulgai, M. xenopi, M. asciaticum, M. aurum, M. smegmatis, M. vaccae, M. fortuitum subsp foruitum, M. fortuitum subsp. Peregrinum, M. chelonae subsp.

Chelonae, M. chelonae subsp. Abscessur, M. genavense, M. tuberculosis, M. tuberculosis $H_{37}R_v$, M. paratuberculosis.

The object of the present invention is to provide a pharmaceutical composition containing 'Mycobacterium w' (Mw) for the treatment of cancer.

Another object of the present invention is to provide a pharmaceutical composition containing 'Mycobacterium w' (Mw) to improve quality of life in patient suffering from cancer.

Yet another object of the invention is to provide a pharmaceutical composition derived from mycobacterium w that are useful for the management of cancer.

Yet another object of the invention is to provide a pharmaceutical composition derived from Mycobacterium w to provide symptomatic relief for patients suffering from cancer.

Yet another object of present invention is to provide a pharmaceutical composition which decreases side effects of standard therapy like radiotherapy, chemotherapy.

Yet another object of present invention is to provide an a pharmaceutical composition containing 'Mycobacterium w' (Mw) which decreases burden of cancer cells/tissues of primary and/or secondary(metastatic), sensitive and/or refractory to conventional treatment.

Yet another object of present invention is to provide a pharmaceutical composition which improves effect of conventional therapies.

## Brief description of the drawings

Figure-1 is X ray non small cell lung cancer before treatment – subject 1

Figure-2 is X ray non small cell lung cancer after treatment –subject 1

Figure-3 is X ray non small cell lung cancer before treatment – subject 2

Figure-4 is X ray non small cell lung cancer after treatment –subject 2

Figure-5 is CT Scan report of patient operated for colorectal cancer with liver metastatisis before treatment

Figure-6 is CT Scan report of patient operated for colorectal cancer with liver metastatisis after treatment

## DETAILED DESCRIPTION OF THE INVENTION

In accordance with the invention the composition of a pharmaceutical composition the method of preparation, HPLC characteristic its safety and tolerability, methods of use and outcome of treatments are described in following examples. The following are illustrative examples of the present invention and scope of the present invention should not be limited by them.

**Example 1. The pharmaceutical compositions:**

A. Each dose of 0.1 ml of therapeutic agent contains:

| | |
|---|---|
| Mycobacterium w., (heat killed) | $0.50 \times 10^9$ |
| Sodium Chloride I. P. ... | 0.90% w/v |
| Tween 80 | 0.1% w/v |
| Thiomerosal I. P. ... | 0.01% w/v |
| (As a Preservative) | |
| Water for injection I. P. | q. s. to 0.1 ml |

B. Each dose of 0.1 ml of therapeutic agent contains:

| | |
|---|---|
| Mycobacterium w., (heat killed) | $0.50 \times 10^9$ |
| Sodium Chloride I. P. ... . | 0.90% w/v |
| Triton x 100 | 0.1% w/v |
| Thiomerosal I. P. ... . | 0.01% w/v |
| (As a Preservative) | |
| Water for injection I. P. | q. s. to 0.1 ml |

C. Each dose of 0.1 ml of therapeutic agent contains:

| | |
|---|---|
| Mycobacterium w., (heat killed) | $0.50 \times 10^9$ |
| Sodium Chloride I. P. ... . | 0.90% w/v |
| Thiomerosal I. P. ... . | 0.01% w/v |
| (As a Preservative) | |
| Water for injection I. P. | q. s. to 0.1 ml |

D. Each dose of 0.1 ml of therapeutic agent contains

Extract of Mycobacterium w after sonication from $1x10^{10}$ Mycobacterium w

| | |
|---|---|
| Sodium Chloride I. P. ... . | 0.90% w/v |
| Thiomerosal I. P. ... . | 0.01% w/v |
| (As a Preservative) | |
| Water for injection I. P. | q. s. to 0.1 ml |

E. Each dose of 0.1 ml of therapeutic agent contains

Methanol Extract of $1x10^{10}$ Mycobacterium w

| | |
|---|---|
| Sodium Chloride I. P. ... . | 0.90% w/v |
| Thiomerosal I. P. ... . | 0.01% w/v |
| (As a Preservative) | |
| Water for injection I. P. | q. s. to 0.1 ml |

F. Each dose of 0.1 ml of therapeutic agent contains

Chloroform Extract of $1x10^{10}$ Mycobacterium w

| | |
|---|---|
| Sodium Chloride I. P. ... . | 0.90% w/v |
| Thiomerosal I. P. ... . | 0.01% w/v |
| (As a Preservative) | |
| Water for injection I. P. | q. s. to 0.1 ml |

G. Each dose of 0.1 ml of therapeutic agent contains

Acetone Extract of $1x10^{10}$ Mycobacterium w

| | | |
|---|---|---|
| Sodium Chloride I. P. | ... . | 0.90% w/v |
| Thiomerosal I. P. | ... . | 0.01% w/v |
| (As a Preservative) | | |
| Water for injection I. P. | | q. s. to 0.1 ml |

H. Each dose of 0.1 ml of therapeutic agent contains

| | | |
|---|---|---|
| Ethanol Extract of $1x10^{10}$ Mycobacterium w | | |
| Sodium Chloride I. P. | ... . | 0.90% w/v |
| Thiomerosal I. P. | ... . | 0.01% w/v |
| (As a Preservative) | | |
| Water for injection I. P. | | q. s. to 0.1 ml |

I. Each dose of 0.1 ml of therapeutic agent contains

| | | |
|---|---|---|
| Liticase Extract of $1x10^{10}$ Mycobacterium w | | |
| Sodium Chloride I. P. | ... . | 0.90% w/v |
| Thiomerosal I. P. | ... . | 0.01% w/v |
| (As a Preservative) | | |
| Water for injection I. P. | | q. s. to 0.1 ml |

J. Each dose of 0.1 ml of therapeutic agent contains

Mycobacterium w (heat killed) $0.5x10^{7}$
Extract of mycobacterium w obtained $1x10^{3}$ Mycobacterium w by disruption, solvent extraction or enzymatic extraction.

| | | |
|---|---|---|
| Sodium Chloride I. P. | ... . | 0.90% w/v |
| Thiomerosal I. P. | ... . | 0.01% w/v |
| (As a Preservative) | | |
| Water for injection I. P. | | q. s. to 0.1 ml |

**Example 2. The Process of preparing a pharmaceutical composition**

A. Culturing of Mycobacterium w.

i) Preparation of calture medium.

Mycobacterium w is cultured on solid medium like L J medium or liquid medium like middle brook medium or sauton's liquid medium.

For better yield middle brook medium is enriched. It can be preferably enriched by addition of glucose, bactotryptone, and BSA. They are used in ratio of 20:30:2 preferably.

The enrichment medium is added to middle brook medium. It is done preferably in ratio of 15:1 to 25:1 more preperably in ratio of 20:1.

ii) Bioreactor operation

a) Preparation of vessel:

The inner contact parts of the vessel (Joints, mechanical seals, o-ring/gasket grooves, etc.) should be properly cleaned to avoid any contamination. Fill up the vessel with 0.1 N NaOH and leave as such for 24 H to remove pyrogenic materials and other contaminants. The vessel is then cleaned first with acidified water, then wit ordinary water. Finally, the vessel is rinsed with distilled water (3 times) before preparing medium.

b) Sterilization of bioreactor

The bioreactor containing 9L distilled water is sterilized with live steam(indirect). Similarly the bioreactor is sterilized once more with Middlebrook medium. The other addition bottles, inlet/outlet air filters etc. are autoclaved (twice) at 121$^0$C for 15 minutes. Before use, these are dried at 50$^0$ C oven.

c) Environmental parameter

i. Temprature: 37± 0.5$^0$ C

ii. pH : 6.7 to 6.8 initially.

B. Harvesting and concentrating

It is typically done at the end of 6$^{th}$ day after culturing under aseptic condition. The concentration of cells (palletisation) is done by centrifugation.

C. Washing of cells

The pallet so obtained is washed minimum three times with normal saline. It can be washed with any other fluid which is preferably isotonic.

D. Adding pharmaceutically acceptable carrier.

Pyrogen free normal saline is added to pallet. Any other pyrogen free isotonic fluid can be used as a pharmaceutical carrier. The carrier is added in amount so as get to desired concentration of active in final form.

E. Adding preservative

To keep the product free from other contaminating bacteria for its self life preservative is added. Preferred preservative is thiomesol which is used in final concentration of 0.01 % w/v.

F. Terminal Sterilization

Terminal sterilization can done by various physical methods like application of heat or ionizing radiation or sterile filtration.

Heat can be in the form of dry heat or moist heat. It can also be in the form of boiling or pasturisation.

Ionizing radiation can be ultraviolet or gamma rays or mircrowave or any other form of ionizing radiation.

It is preferable to autoclave the final product.

This can be done before after filling in a final packaging.

G. Quality Control

i. The material is evaluated for purity, sterility.

ii. The organisms are checked for acid fastness after gram staining.

iii. Inactivation test : This is done by culturing the product on L J medium to find out any living organism.

iv. Pathogenicity and/or contamination with pathogen.

The cultured organisms are infected to Balb/c mice.
None of the mice should die and all should remain healthy and gain weight. There should not be any macroscopic or microscopic lesions seen in liver, lung spleen or any other organs when animals are killed upto 8 weeks following treatment.

v. Biochemical Test:

The organism is subjected to following biochemical tests:

a) Urease

b) Tween 80 hydrolysis

c) Niacin test

d) Nitrate reduction test

The organism gives negative results in urease, tween 80 hydrolysis and niacin test. It is positive by nitrate reduction test.

H. Preparation of constituents of Mycobacterium w.

The constituents of Mycobacterium w can be prepared for the purpose of invention by:

I. Cell disruption

II. Solvent extration

III. Enzymatic extraction.

The cell disruption can be done by way of sonication or use of high pressure fractionometer or by application of osmotic pressure ingredient.

The solvent extraction can be done by any organic solvent like chloroform, ethanol, methanol, acetone, phenol, isopropyl alcohol, acetic acid, urea, hexane etc.

The enzymatic extraction can be done by enzymes which can digest cell wall/membranes. They are typically proteolytic in nature. Enzyme liticase and pronase are the preferred enzymes. For the purpose of invention cell constituents of Mycobacterium w can be used alone in place of mycobacterium w organisms or it can be added to the product containing mycobacterium w.

Addition cell constituents results in improved efficacy of the product.

**Example 3. Characteristics of constituents of Mycobacterium w by HPLC analysis.**

The constituents of mycobacterium w. used for the purpose of invention when subjected to HPLC analysis gives a single peak at 11 minutes. No other significant peaks are found beyond. The peak is homogenous and devoid of any notch suggesting homogeneity of material obtained

HPLC analysis was done using a waters system high performance liquid chromatography apparatus

| | |
|---|---|
| Column: | Novapak c1860A, 4μm, 3.9 x 150mm. |
| The guard column: | Novapak c 18 |
| Column Temperature: | $30^0$ c |
| Flow rate: | 2.5 ml/min |

Injection volume: 25μL.
Mobile phase:
Solvent A: HPLC grade methanol.
Solvent B: HPLC grade methylene chloride
Binary gradient:
The HPLC gradient initially comprised 98%(v/v) methanol (solvent B).
The gradient was increased linearly to 80%.
A and 20% B at one minute; 35% A and 65% B at 10 minutes, held for 5 seconds and then decreased over 10 seconds back to 98% A and 2% B.

**Example 4. Management of cancer refractory to standard treatment.**

CASE 1:
A 70 year old female suffering from multiple mycloma was receiving malphalan and prednisolone a therapy for 5 years. The desease recurred with bone pain. Her general condition was poor and she was berdridden. Her hemoglobin was reduced to 5.5.gm. She was put on intradermal injection of a pharmaceutical composition injection of a pharmaceutical composition containing mycobacterium w as per present invention. It was given as 0.1 ml intradermally over deltoid region at the interval of one weel. At the end of 3 months she is symptoms general condition has improved drastically and she is able to walk on her own. Her hemoglobin value has risen to 7.7 gm/dl from 5.5 gm/dl in absence of any specific treatment on anaemia.

CASE 2

A 50 year old postmenopausal woman under went lumpectomy for a fumigating mass in her last breast (carcinoma breast $T_4$, $N_1$ $M_1$). The tumor was hormone independent and receptor status for estrogen and progesterone was negative. Following surgery she developed cough and breathlessness. It was found to be due to large metastatic lesion in her chest. A pharmaceutical composition as per present invention was added to her therapy. At the end of three months, there was a remarkable improvement in her cough and breathlessness. X-ray chest showed 25% decrease in size of metastatic lesion. The mycobacterium w containing pharmaceutical cxomposition as per present invention was administered intradermally over deltoid region.

CASE 3

A 68 year old male suffering from carcinoma esophagus-midthird had received radiotherapy and was on chemotherapy (one cycle completed) He developed dysphagia due to progress of disease. He also had meutropenia with fall in total WBC count. Therapy with a pharmaceutical composition was started. It resulted in improvement in his symptoms gradually. A the end of three months. The swallowing became normal with improvement in general condition and

normalization of WBC count. The pharmaceutical composition as per present invention was given as 0.1ml intradermally at weekly interval 2nd dose was delayed and administered at the interval of 15 days instead of 1 week. It comprised of 0.3ml instead of 0.1 ml.

In case 1 and 3 improvement was seen inspite of absence of chemotherapy while in case 2 chemotherapy(FAC) also continued.

Thus this cases illustrates that pharmaceutical compositions containing Mycobacterium w(Mw) as per present invention are useful in treatment of cancer which are refractory to standard therapy. Their use is associated with amelioration of symptoms, improvement in general well being and quality of life, improvement in other associated conditions like anemia, neutropenia.

**Example 5. Effect if ogarnaceutical composition on cancer when used alone.**

Superficial bladder cancer presents as hematuria. It is amenable to various forms of therapy. Drugs used to achieve remission are given intravesically e.g. doxorubinocin or BCG.

In four patients with superficial bladder cancer diagnosed cystoscopically pharmaceutical composition as per present invention was given intradermally. It was given as 0.1ml every month. By six weeks (after two injections) everybody became asymptomatic. Eight weeks later cystoscopy was performed. Surprisingly it was found that there was absence of any detectable lesion cystyoscopically. Six months followup did not reveal any recurrence of symptoms. Cystoscopy also revealed normal bladder mucosa with absence of detectable lesion.

Thus findings are suggestive of effect of pharmaceutical composition containing mycobacterium w as effective therapy in management of bladder cancer when given intradermally over deltoid region.

**Example 6. Effect of Mycobacterium w when radiotherapy is not adequate.**

Muscle invasive bladder cancer.

Muscle invasive bladder cancer ($T_4$) can be managed by radical cystectomy. However it is desirable to preserve bladder. Radiotherapy and/or chemotherapy are not adequate in achieving local control/remission of disease.

Five patients with muscle invasive bladder cancer were treated by intradermal injection of Mycobacterium w over both deltoid. The intradermal Mycobacterium

w was repeated every month on any on deltoid for six months. All received standard radiotherapy for a total of 71 gy.

At the end of two months all were symptom free. Cystoscopy and computerized axial tomography(T scan) failed to reveal any detectable lesion suggesting complete remission of disease. All are disease free after a followup of 8 months or longer after beginning therapy.

Thus mycobacterium w is effective in achieving complete remission and maintaining it.

No side effects were noticed by any of the patients.

**Example 7. Effect of Mycobacterium w when chemotherapy is not adequate.**

Non small cell lung cancer.

Non small cell lung cancer is difficult to manage. It usually does not respond well to chemotherapy or radiotherapy. The response rate is inversely proportionate to extent of disease. In disease with extent $T_4$ surgery is not indicated and chemotherapy and/or radiotherapy has hardly any effect and carries poor prognosis.

Thus findings of this study suggests that mycobacterium w has significant effect on difficult to treat cancer.

**Example 8. Effect of Mycobacterium w containing therapy on Quality of life and side effects of chemotherapy**

**a) Carcinoma breast with bone metastasis.**

In a controlled study involving 20 patients with a breast cancer and bone metastasis effect of mycobacterium w was evaluated. All patients received chemotherapy in the form of cyclophosphamide, adriamycin and 5-fluorouracil. Mycobacterium w containing compositions were given as intradermal injections of 0.1 ml every wekk for two months followed by every 15 days for two months and monthly for two months for a total duration six months. 10 patients of 20 randemly received it while remaining 10 were kept as controls.

None of the patients in treatment group developed diarrhoea and vomiting compared to 8 of 10 patients in control group. Mucositis was seen in 1 patient in treatment group compared to 5 patients in control group. Bone marrow depression as manifested by leucopenia, thrombocytopenia and anemia was seen in 2 patients in treatment group and 6 patients in control group. There was

in crease in weight in 3 patients in treatment group while none of the patients showed increase in weight. All patients had remarkable improvement in quality of life in treatment group which was not seen in any one in control group.

**b) Head and neck cancer.**

In a controlled study in 20 patients with histologically proven advanced head and neck cancer with minimum of 6 months expectancy effect of mycobacterium w was evaluated. Each patient received chemotherapy containing cisplatin and 5-FU. Mycobacterium w was given to randomly selected 10 patients as 0.1 ml intradermally over deltoid region every 15 days for 3 months. The first dose was given as 0.2 ml divided over two deltoid region.

The chemotherapy induced hematological side effects resulted in postponement of chemotherapy in 2 out of 10 patients in treatment group and 5 out of 10 patients in control group.

Mucositis was evident on day 15 after chemotherapy. It was seen in 2 patients in treatment group and 6 patients in control group.

Nausea/vomiting was seen in all patients in control group while in none in treatment group.

Thus use of mycobacterium w was useful in reducing side effects of chemotherapy.

**Example 9. Use of Mycobacterium w in terminally sick patients with cancer.**

A 65 year old male patient was diagnosed to have carcinoma pancreas with metastasis in liver and lung. He was judged to be terminally ill with incurable cancer was not offered any treatment. He developed extensive cough and breathlessness and was unable to sleep. He was administered Mycobacterium w 0.3 ml intradermally biweekly. Within 10 day his cough was controlled and general condition showed improvement. He started doing all his routine work by himself and started getting normal sleep. If for some reason administration of Mycobacterium w was delayed beyond 4 days there used to be recurrence of cough and disturbances in sleep. He lived for 14 weeks after therapy and died natural death. X-ray chest taken before and two months after showed some improvement in lesion. He did not receive any other therapy all throughout.

Thus Mycobacterium w was useful in ameliorating symptoms in terminally ill patient due to cancer.

**EDITORIAL NOTE**

**APPLICATION NUMBER - 2002348738**

**This specification does not contain a page(s) "15" (to "18").**

We Claim:

1. A method of treating cancer comprising administration of a formulation which is prepared using mycobacterium w or a pharmaceutical composition obtained from mycobacterium w alone or in combination and also with or without adjuvants to a subject who has been suffering from cancer.
2. A product created from the method as claimed in claim 1 contain mycobacterium w is killed mycobacterium w.
3. The Mycobacterium w as claimed in claim 1 or 2 is killed by physical method like heat radiation most preferably by heat in form of autoclaving.
4. A product created from the method as claimed in claim 1 is obtained from mycobacterium w by sonication.
5. A product created from the method as claimed in claim 1 is obtained from mycobacterium w by extraction.
6. A product created from the method as claimed in claim 1 or a product according to claim 5 is obtained from mycobacterium w is extracted by organic solvents.
7. A product created from the method as claimed in claim 1, or a product according to claim 6 is extracted using solvent selected from chloroform, ethanol, methanol, acetone, phenol, isopropyl alcohol, acetic acid, urea, Hexane and like.
8. The adjuvants as claimed in claim 1 is selected from mineral oil, mineral oil and surfactant, Ribi adjuvant, Titer-max, syntax adjuvant formulation, aluminium salt adjuvant, nitrocellulose adsorbed antigen, formulation, aluminium salt adjuvant, nitrocellulose adsorbed antigen, immune stimulating complexes, Gebru adjuvant, super carrier, elvax 40w, L-tyrosine, monatanide (manide -oleate compound), Adju prime, Squalene, Sodium phthalyl lipopoly saccharide, calcium phosphate, saponin, melanoma antigen, muramyl dipeptide (MDP and like.
9. A method according to claim 1 wherein the formulation contains surfactant.
10. A method according to claim 9 wherein the surfactant can be a Tween 80.
11. A method according to claim 9 or 10 wherein the amount of surfactant is up to 0.4% preferably 0.1%.

12. A method according to claim 1 wherein the formulation containing mycobacterium w or obtained from mycobacterium w or combination of both with or without adjuvants helps in amelioration of symptoms of cancer.
13. A method according to claim 1 wherein the formulation containing mycobacterium w or obtained from mycobacterium w or combination of both with or without adjuvants are capable of causing regression or even complete control of cancer.
14. The Mycobacterium as claimed in any one of claims 1 to 6 is a non-pathogenic, fast growing cultivable, atypical mycobacterium, with biochemical properties and growth characteristics resembling those belonging to Runyons group IV class of Mycobacteria in its metabolic growth properties but is not identical to those strains currently listed in this group.
15. Mycobacterium w as claimed in claim 1 is urease negative, does not hydrolyse tween 80, does not produce niacin, provides strong positive response to nitrate reduction test.
16. The method as claimed in claim 1 for management of cancer is effective when used alone or combination with other modalities of cancer treatment like chemotherapy, radiotherapy, surgery.
17. The method as claimed in claim 1 for management of cancer is effective in improving quality of life in patient who are suffering from cancer.
18. The improvement in quality of life as claimed in claim 14 is obtained in absence as well as presence of other modes of treatment.
19. The method as claimed in claim 1 for management of cancer is effective in amelioration of symptoms associated with cancer.
20. The method as claimed in claim 1 for management of cancer is effective in decreasing the burden of cancer tissue.
21. The decrease in burden of cancer tissue as claimed in claim 17 is obtained in absence as well as presence of other modes of therapy.
22. The cancerous tissue as claimed in claim 17 can be a primary or a secondary (metastatic) lesion.
23. The method as claimed in claim 1 is effective in reducing side effects of other cancer therapies like radiotherapy, chemotherapy.
24. The administration of formulation as claimed in claim 1 is by parental route.

25. The administration as claimed in claim 1 and 17 is by intramuscular subcutaneous, intradermal route and like but preferably by intradermal route.
26. The amount of mycobacterium w administered at a time to a subject as claimed in claim 1 is equal to or more than $1 \times 10^5$ mycobacterium w.
27. The amount of mycobacterium w administered at a time to a subject as claimed in claim 1 is equal to or more than $10^7$ mycobacterium w.
28. The amount of mycobacterium w administered at a time to a subject as claimed in claim 1 is most preferably $1 \times 10^8$ to $1 \times 10^{10}$ mycobacterium w.
29. The process of manufacturing a pharmaceutical composition useful for management of cancer comprises of incorporating cells of mycobacterium w along with pharmaceutically acceptable carrier and optionally a preservative in a single formulation wherein cells of mycobacterium w are not alive.
30. The pharmaceutically acceptable carrier as claimed in claim 1 is added in a way so as to have more than or equal to $1 \times 10^5$ mycobacterium w in a unitary dosage, more preferably equal to or more than $1 \times 10^7$ mycobacterium w in unitary dosage most preferably between $1 \times 10^8$ to $1 \times 10^9$ cells of mycobacterium w in a unitary dosage form.
31. The process of manufacturing a pharmaceutical composition useful for management of cancer comprising the steps of incorporating disrupted cells of mycobacterium w along with pharmaceutically acceptable carrier and optionally a preservative.
32. The process of manufacturing a pharmaceutical composition useful for management of cancer comprising the steps of incorporating solvent extraction of mycobacterium w along with pharmaceutically acceptable carrier and optionally a preservative.
33. The process of manufacturing a pharmaceutical composition useful for management of cancer comprising of incorporating enzymatic extraction of mycobacterium w along with pharmaceutically acceptable carrier and optionally a preservative.
34. The process of manufacturing a pharmaceutical composition useful for management of cancer comprising admixing product of claim 1 with product of any one of claims 31, 32 and 33

35. The process of manufacturing a pharmaceutical composition useful for management of cancer comprise of adding adjuvant to product of any one of claims 1, 4, 6, 8 and 10.
36. A product created from the method of claim 1 and including an adjuvant selected from the group consisting of mineral oil, mineral oil and surfactant, Ribi adjuvant, Titer-max, syntax adjuvant formulation, aluminium salt adjuvant, nitrocellulose adsorbed antigen, immune stimulating complexes, Gebru adjuvant, super carrier, elvax 40w, L-tyrosine, monatanide (manide-oleate compound), Adju prime, Squalene, Sodium phthalyl lipopoly saccharide, calcium phosphate, saponin, melanoma antigen, muramyl dipeptide (MDP) and like.
37. The process as claimed in claim 29 wherein the preservative is thiomersol and is added to have final concentration of 0.01% w/v.
38. The process as claimed in claim 31 wherein disruption of the mycobacterium w is done by sonication or high pressure fractionometer.
39. The process as claimed in claim 32 wherein solvent extraction is done by using a solvent selected from the group consisting of chloroform, ethanol, methanol, acetone, phenol or isopropyl alcohol, acetic acid, urea, etc.
40. The enzymes used for enzymatic extraction of cells of mycobacterium w is selected from lyticase and/or pronase.
41. The process of manufacturing a pharmaceutical composition useful for management of cancer comprising admixing product of claim 37 with product of any one of claims 31 to 33.
42. The process of manufacturing a pharmaceutical composition useful for management of cancer comprised of adding adjuvant to product of any one of claims 31, 32, 34 and 40.
43. The adjuvant as claimed in claim 42 is selected from mineral oil, mineral oil and surfactant, Ribi adjuvant, Titer-max, syntax adjuvant formulation, aluminium salt adjuvant, nitrocellulose adsorbed antigen, immune stimulating complexes, Gebru adjuvant, super carrier, elvax 40w, L-tyrosine, monatanide (manide-oleate compound), Adju prime, Squalene, Sodium phthalyl lipopoly saccharide, calcium phosphate, saponin, melanoma antigen, muramyl dipeptide (MDP) and like.

44. A pharmaceutical composition prepared according to any one of claims 29 to 33 or claims 37 to 43 when administered to a subject suffering from Cancer in therapeutic dosage results in amelioration of his symptoms.
45. A pharmaceutical composition prepared according to any one of claims 29 to 33 or claims 37 to 43 when administered to a subject suffering from Cancer in therapeutic dosage results in improvement in quality of life.
46. A pharmaceutical composition prepared according to any one of claims 29 to 33 or claims 37 to 43 when administered to a subject suffering from Cancer in therapeutic dosage is useful in reducing side effects of other therapy like chemotherapy and or radiotherapy used in management of cancer.
47. A pharmaceutical composition prepared according to any one of claims 29 to 33 or claims 37 to 43 when administered to a subject suffering from Cancer in therapeutic dosage is useful in improving results of chemotherapy and/or radiotherapy used in management of cancer.
48. A pharmaceutical composition prepared according to any one of claims 29 to 33 or claims 37 to 43 when administered to a subject suffering from Cancer in therapeutic dosage is useful in achieving control of cancer
49. A pharmaceutical composition prepared according to any one of claims 29 to 33 or claims 37 to 43 when administered to a subject suffering from Cancer in therapeutic dosage is useful in reducing burden of cancer when used alone or in combination of other therapies.
50. A Pharmaceutically acceptable carrier used in the process as claimed in any one of claims 29, 31 to 33 or 41 or 42 contains surfactant.
51. The surfactant as claimed in claim 50 is selected from Tween 80 or triton x 100.
52. The concentration of surfactant as claimed in claim 50 or 51 is up to 0.4% preferably 0.1%.
53. The use of mycobacterium w or constituents of mycobacterium w in the preparation of a pharmaceutical composition for use in treating or managing cancer.
54. The use of mycobacterium w or constituents of mycobacterium w in the preparation of a pharmaceutical composition for use in decreasing the burden of cancer tissue.

55. The use as claimed in any one claims 53 or 54 wherein the pharmaceutical composition is for use in improving the cancer treating effect of radiotherapy or chemotherapy.
56. The use as claimed in any one of claims 53 to 55 wherein the pharmaceutical composition is for use in reducing the side-effects of radiotherapy or chemotherapy.
57. The use as claimed in claim 56 wherein the side effects are hematological side effects.
58. The use as claimed in claim 57 wherein the hematological side effects are reduced to avoid postponement of chemotherapy.
59. The use as claimed in claim 56 wherein the side effects are leucopenia, thrombocytopenia, anaemia, nausea, vomiting or mucositis.
60. The use as claimed in any one of the preceding claims wherein the mycobacterium w is dead mycobacterium w.
61. The use as claimed in claim 60 wherein the mycobacterium w has been killed by a physical method.
62. The use as claimed in claim 61 wherein the physical method is the application of heat.
63. The use as claimed in claim 62 wherein the heat is applied by means of autoclaving.
64. The use as claimed in any one of claims 53 to 59 wherein the constituents of mycobacterium w have been obtained by sonication.
65. The use as claimed in any one of claims 53 to 59 wherein the constituents of mycobacterium w have been obtained by extraction.
66. The use as claimed in claim 65 wherein the constituents of mycobacterium w has been extracted by organic solvents.
67. The use as claimed in claim 66 wherein the organic solvents are selected from chloroform, ethanol, methanol, acetone, phenol, isopropyl alcohol, acetic acid, area and hexane.
68. The use as claimed in any one of claims 53 to 67 wherein the pharmaceutical composition further comprises one or more adjuvants.
69. The use as claimed in any one of claims 53 to 68 wherein the pharmaceutical composition further comprises a surfactant.

70. The use as claimed in claim 69 wherein the surfactant is polyoxyethylene (20) sorbitan monooleate.

71. The use as claimed in claim 69 or claim 70 wherein the pharmaceutical composition comprises a surfactant in an amount up to 0.4% by weight/volume of the pharmaceutical composition.

72. The use as claimed in claim 71 wherein the surfactant is present in an amount up to 0.1% by weight/volume of the pharmaceutical composition.

73. The use as claimed in any one of claims 53 to 72 wherein the mycobacterium w is urease negative, does not hydrolyse polyoxyethylene (20) sorbitan monooleate, does not produce niacin, provides a strong positive response to nitrate reduction tests.

74. The use as claimed in any one of claims 53 to 73 wherein the pharmaceutical composition is for administration alone or in combination with other modes of therapy.

75. The use as claimed in any one of claims 53 to 74 wherein the pharmaceutical composition is for administration by parental route.

76. The use as claimed in any one of claims 53 to 75 wherein the pharmaceutical composition is for administration by intramuscular, subcutaneous or intradermal route.

77. The use as claimed in any one of claims 53 to 76 wherein the pharmaceutical composition is in a unit dosage form comprising at least $10^5$ mycobacterium w.

78. The use as claimed in 77 wherein the pharmaceutical composition is in a unit dosage form comprising at least $10^7$ mycobacterium w.

79. The use as claimed in claim 78 wherein the pharmaceutical composition is in a unit dosage form comprising from $10^8$ to $10^{10}$ mycobacterium w.

80. The use as claimed in any one of claims 53 to 79 wherein the pharmaceutical composition further comprises a preservative.

81. The use as claimed in any one of claims 53 to 80 wherein the cancer is a primary or a secondary (metastatic) lesion.

82. The use of mycobacterium w. or constituents of mycobacterium w. in the preparation of a pharmaceutical composition for use in treating or managing cancer substantially as herein described and with reference to the Examples.

83. A method of treating cancer substantially as herein described with references to any one or more of the Examples.
84. A formulation compound or composition substantially a herein described with references to any one or more of the Examples.
85. A process for manufacturing a pharmaceutical composition, or compound or formulation, said process being substantially as herein described with references to any one or more of the Examples.

Dated this 9th day of October 2007

Bakulesh Mafatial KHAMAR

By
FRASER OLD & SOHN
Patent Attorney for the Applicant

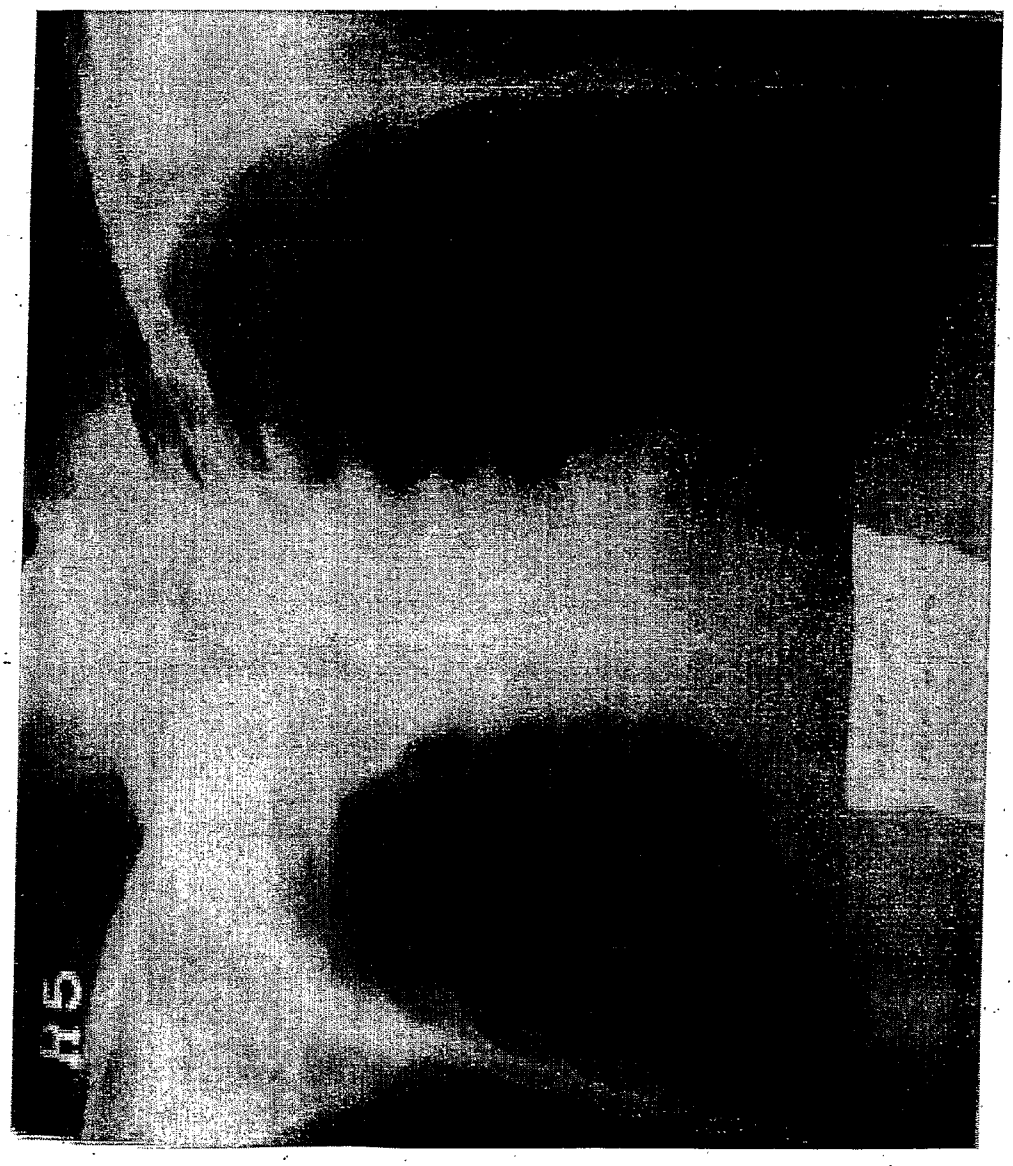

Figure-1 is X ray non small cell lung cancer before treatment – subject 1

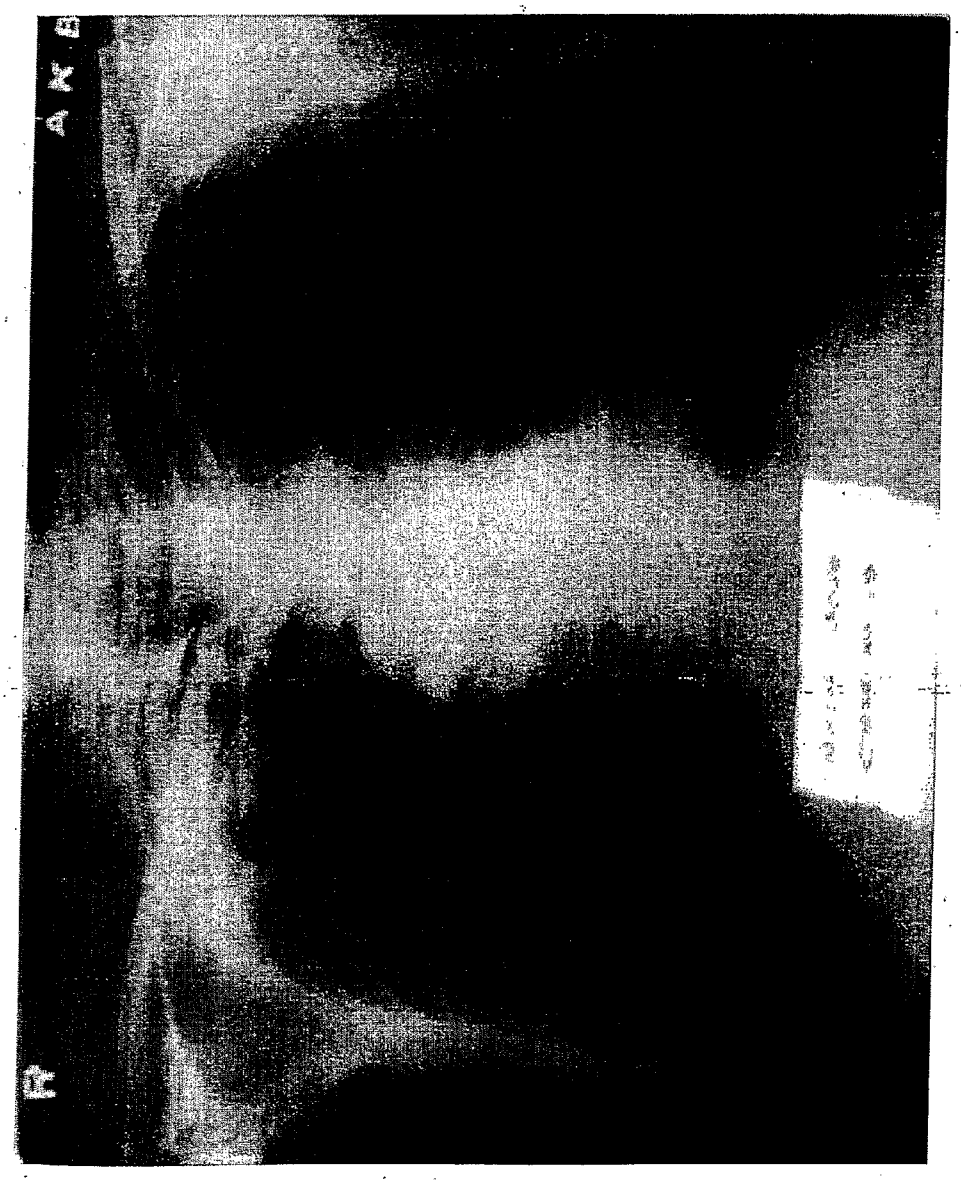

Figure-2 is X ray non small cell lung cancer after treatment –subject 1

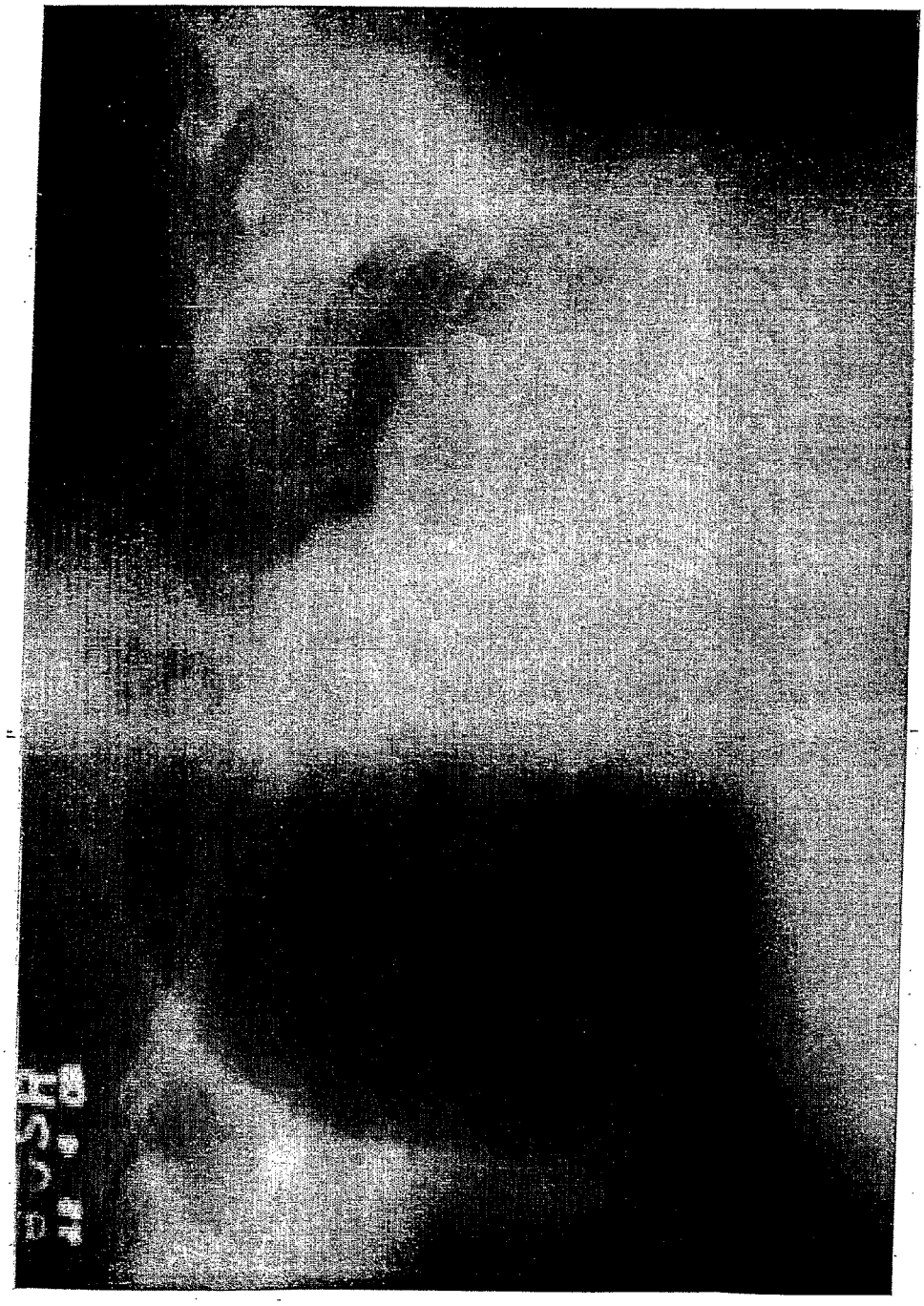

Figure-3 is X ray non small cell lung cancer before treatment — subject 2

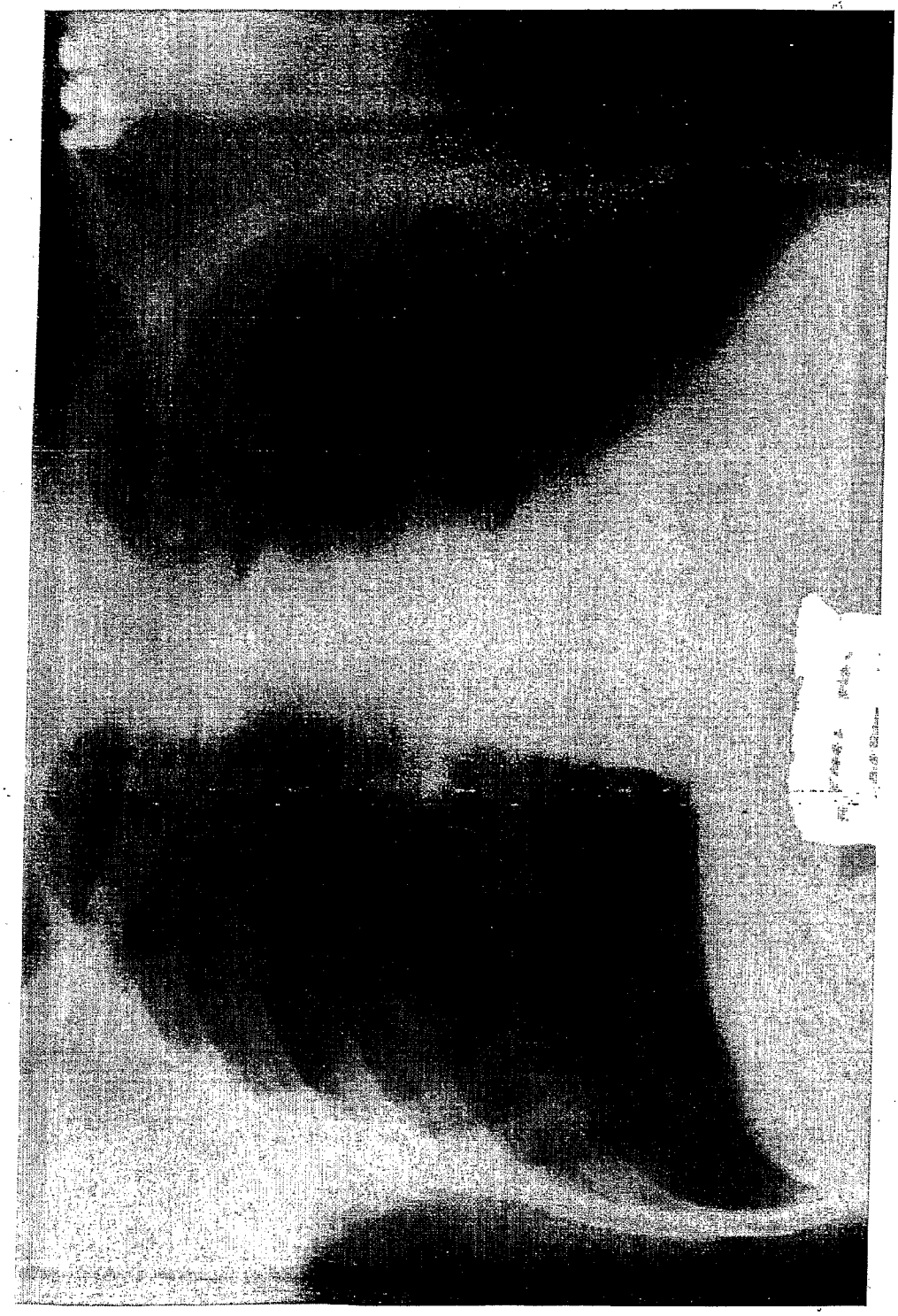

Figure-4 is X ray non small cell lung cancer after treatment –subject 2

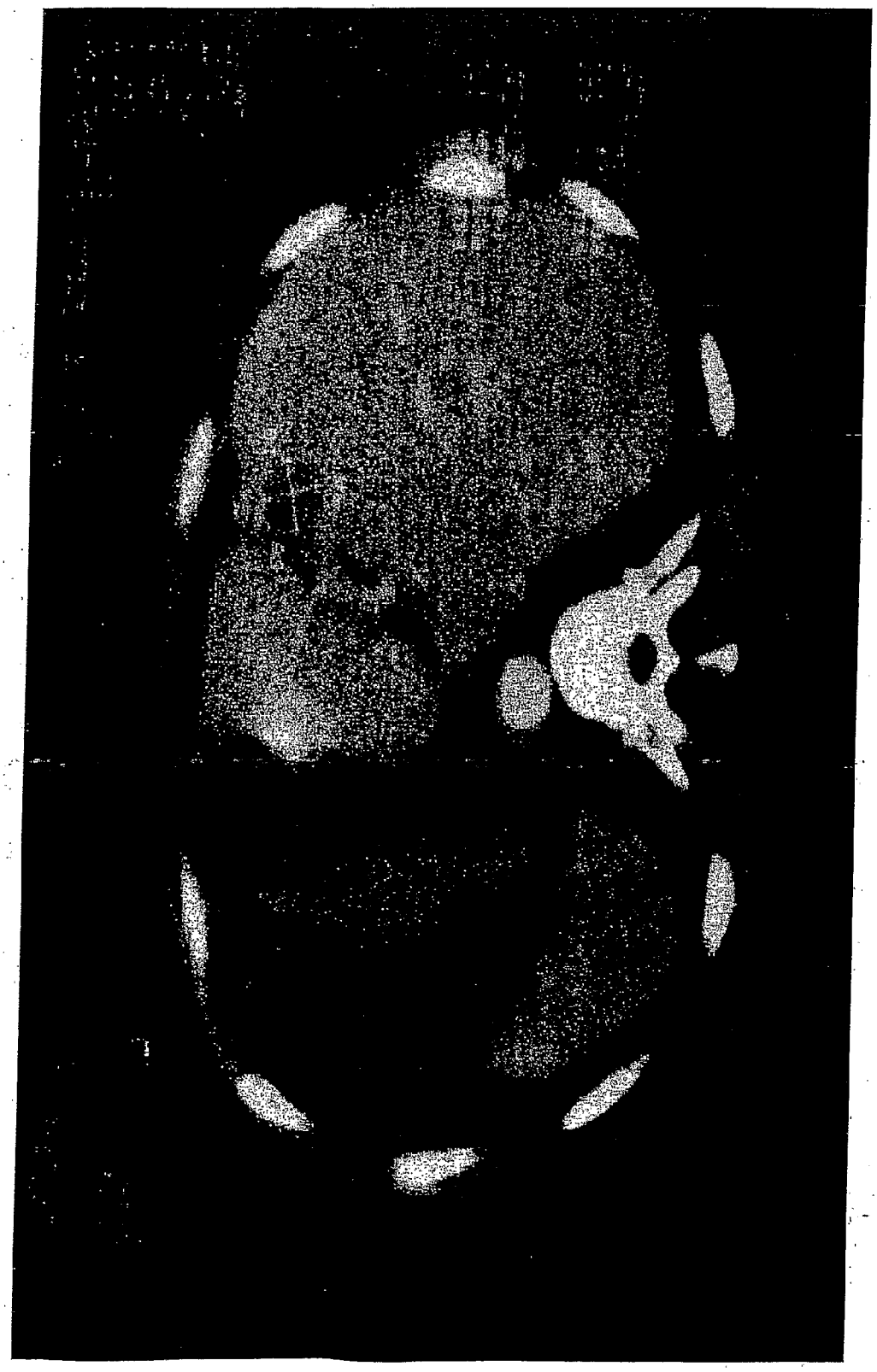

Figure-5 is CT Scan report of patient operated for colorectal cancer with liver metastatisis before treatment ( 38 x38 mm)

Figure-6 is CT Scan report of patient operated for colorectal cancer with liver metastatisis after treatment (20 x 20 mm)