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COMPREHENSIVE ANALYSIS OF MESOTHELIN GENE IN ASBESTOS RELATED HUMAN MESOTHELIOMA

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ABSTRACT

Mesothelioma was tend to be aggressive tumors that arise from the serosal surface cells lining the pleura, peritoneum and pericardium. The majority (80%) of these tumors are associated with exposure to asbestos fibers, either in the environment or work place. Mesothelin gene, was a differentiation antigen, glycosylphosphatidylinositol-linked cell-surface glycoprotein, that was present on the surface of normal mesothelium and was over expressed in many patients with epithelial ovarian cancer and malignant mesothelioma. Our research was focusing on MSLN gene hence it was a promising candidate for tumor specific therapy and it was used as a biomarker also. The goal of present study was to clarify in which condition it gets over expressed. Mesothelin family of proteins found that patients with malignant mesothelioma had higher levels of serum mesothelin related peptide (SMR) than the healthy controls. It provided an increased understanding of mesothelin gene, and further study of this gene may ultimately provide new opportunities for the development of therapeutics. The over expression of the gene was due to the proteolysis and this leads to the release of the soluble peptide in to the serum. An extensive analysis of the Mesothelin gene including modeling of its structure may prove useful in designing a drug for Mesothelioma treatment. Further extensive research work on this protein will leads to a new milestone in mesothelioma research.

Keywords: *Mesothelin gene, Asbetos, Serum Peptide, Glycoprotein, Proteolytic cleavage*

1. INTRODUCTION

Cancer was a group of diseases in which cells are aggressive (grow and divide without respect to normal limits), invasive (invade and destroy adjacent tissues), and/or metastatic (spread to other locations in the body). These three malignant properties of cancers differentiate them from benign tumors, which are self-limited in their growth and do not invade or metastasize (although some benign tumor types are capable of becoming malignant). Cancer may affect people at all ages, even fetuses, but risk for the more common varieties tends to increase with age. Cancer causes about 13% of all deaths.

Chemicals are thought to promote cancer through their stimulating effect. Substances that cause DNA mutations are known as mutagens, and mutagens that cause cancers are known as carcinogens. Particular substances have been linked to specific types of cancer. Tobacco smoking was associated with lung cancer and bladder cancer. Prolonged exposure to asbestos fibers was associated with Mesothelioma. (WHO).

Mesotheliomas tend to be aggressive tumors that arise from the serosal surface cells lining the pleura, peritoneum and pericardium. The majority (80%) of these tumors are associated with exposure to asbestos fibers, either in the environment or work place. Although asbestos has been banned for use in most developed countries and asbestos abatement programs have been in place for the past several decades, over 2,000 cases are diagnosed in the United States each year. This was due to the long latency period from time of

exposure to development of mesothelioma (20 to 40 years). Although mesothelioma incidence in the United States peaked in the mid-1990, it was estimated that over 70,000 mesothelioma cases will occur in US males between 2003 and 2054. It must be realized that less than 5% of those exposed to asbestos will develop mesothelioma. (United States Department of Health and Human Services).

In this disease, malignant cells develop in the mesothelium, a protective lining that covers most of the body's internal organs. Its most common site was the pleura (outer lining of the lungs and chestcavity), but it may also occur in the peritoneum (the lining of the abdominal cavity) or the pericardium (a sac that surrounds the heart).Most people who develop mesothelioma have worked on jobs where they inhaled asbestos particles, or have been exposed to asbestos dust and fibre in other ways, such as by washing the clothes of a family member who worked with asbestos, or by home renovation using asbestos cement products. Unlike lung cancer, there was no association between mesothelioma and smoking

Diagnosing mesothelioma was often difficult, because the symptoms are similar to those of a number of other conditions. Diagnosis begins with a review of the patient's medical history. A history of exposure to asbestos may increase clinical suspicion for mesothelioma. A physical examination was performed, followed by chest X-ray and often lung function tests. The X-ray may reveal pleural thickening commonly seen after asbestos exposure and increases suspicion of mesothelioma. A CT (or CAT) scan or an MRI was usually performed.

If a large amount of fluid was present, abnormal cells may be detected by cytology if this fluid was aspirated with a syringe. For pleural fluid this was done by a pleural tap or chest drain, in ascites with an paracentesis or ascitic drain and in a pericardial effusion with pericardiocentesis. While absence of malignant cells on cytology does not completely exclude mesothelioma, it makes it much more unlikely, especially if an alternative diagnosis can be made (e.g. tuberculosis, heart failure).If cytology was positive or a plaque was regarded as suspicious, a biopsy was needed to confirm a diagnosis of mesothelioma. A doctor removes a sample of tissue for examination under a microscope by a pathologist. A biopsy may be done in different ways, depending on where the abnormal area was located. If the cancer was in the chest, the doctor may perform a thoracoscopy. In this procedure, the doctor makes a small cut through the chest wall and puts a thin, lighted tube called a thoracoscope into the chest between two ribs. Thoracoscopy allows the doctor to look inside the chest and obtain tissue samples.

If the cancer was in the abdomen, the doctor may perform a laparoscopy. To obtain tissue for examination, the doctor makes a small opening in the abdomen and inserts a special instrument into the abdominal cavity. If these procedures do not yield enough tissue, more extensive diagnostic surgery may be necessary.

Asbestos has also been shown to mediate the entry of foreign DNA into target cells. Incorporation of this foreign DNA may lead to mutations and oncogenesis by several possible mechanisms:

- Inactivation of tumor suppressor genes
- Activation of oncogenes
- Activation of proto-oncogenes due to incorporation of foreign DNA containing a promoter region
- Activation of DNA repair enzymes, which may be prone to error
- Activation of telomerase
- Prevention of apoptosis

Asbestos fibres have been shown to alter the function and secretory properties of macrophages, ultimately creating conditions which favour the development of mesothelioma. Following asbestos phagocytosis, macrophages generate increased amounts of hydroxyl radicals, which are normal by-products of cellular anaerobic metabolism. However, these free radicals are also known clastogenic and membrane-active agents thought to promote asbestos carcinogenicity. These oxidants can participate in the oncogenic process by directly and indirectly interacting with DNA, modifying membrane-associated cellular events, including oncogene activation and perturbation of cellular antioxidant defences.

Asbestos also may possess immunosuppressive properties. For example, chrysotile fibres have been shown to depress the in vitro proliferation of phytohemagglutinin-stimulated peripheral blood lymphocytes, suppress natural killer cell lysis and significantly reduce lymphokine-activated killer cell viability and recovery. Furthermore, genetic alterations in asbestos-activated macrophages may result in the release of potent mesothelial cell mitogens such as platelet-derived growth factor (PDGF) and transforming growth factor- â (TGF-â) which in turn, may induce the chronic stimulation and proliferation of mesothelial cells after injury by asbestos fibres.

2. TYPES OF MESOTHELIOMA:

The definition of the word mesothelioma was literally cancer of the mesothelium. The mesothelium was the sac that lines and protects vital organs such as the heart and the lungs, and this disease causes the cells of the lining to become abnormal and malignant.

The result of asbestos exposure, mesothelioma comes in three forms: pleural mesothelioma; peritoneal mesothelioma; and pericardial mesothelioma. All three types have a variety of associated symptoms, and there are some symptoms that are common to all three types of the disease. In all cases of the disease, sufferers are unlikely to even realise that there was a problem until many years after they have actually contracted mesothelioma from regular exposure to asbestos.

The symptoms of all types of mesothelioma do not generally manifest for several decades after contraction. This can make the disease difficult to diagnose and all too often was too late to save the patient by the time a diagnosis was made.

1. Pleural mesothelioma:

Pleural Mesothelioma was the most common form of mesothelioma that exists, and in its malignant form was the direct result of exposure to asbestos fibres. You can get benign tumours with mesothelioma, but the malignant form was by far the most common. The breathing in of asbestos fibres was what puts those who work unprotected with asbestos at risk, and the risks can be heightened by smoking as well as exposure to this hazardous fibre.

This form of mesothelioma attacks the lungs and respiratory areas of the body. The cancer attacks the cells and the lining (known as the pleura) of the lungs and ribs. As with other forms of mesothelioma, the symptoms can take twenty or thirty years (sometimes longer) to present themselves following exposure to asbestos, making it impossible for people to realise that they have been affected until it was too late.

There are a number of symptoms for pleural mesothelioma, and like peritoneal mesothelioma, they can appear very non-specific and could be put down to a number of common diseases or illnesses. The long latency period associated with mesothelioma was already the cause of much delay, but the type of symptoms associated with this disease can cause even further delay, even when the onset begins.

Without prior knowledge that you have worked with asbestos and are therefore a high risk patient where mesothelioma was concerned, your doctor could end up testing for a wide range of other diseases such as pneumonia. This waste much needed time, and it was therefore vital that you not only keep your eyes open for any of the associated symptoms but also inform your doctor of your past history of working with asbestos. Armed with this information, the doctor can then start running appropriate tests should the symptoms manifest.

Some of the symptoms that are commonly associated with pleural mesothelioma include: persistent coughing; difficulty swallowing; facial swelling; weight loss; fever; rasping; and coughing up blood. Some patients may also experience shortness of breath, whether they are being active or even when they are resting. This can be caused by the thickening of the pleura due to the spread of the tumour. The thicker the pleura gets, the less space the lungs have to function properly, hence breathing begins to be affected.

Some patients may also experience severe pain in the chest or ribs, and this was caused by the spread of the cancerous cells and the tumour. Should the tumour start to spread outwards, it will affect the chest area as well as the lungs.

Treating pleural mesothelioma was dependant on many things, and it was the doctor that will normally suggest the recommended course of treatment based upon his knowledge and the findings from his diagnostic tests. Factors such as the patient's age, medical history, general well being, and even weight, are taken into account. Other factors that have to be considered include: how far the cancer has spread; and where the cancer is.

The treatments for the different types of mesothelioma are more or less the same in most cases, but obviously they are targeted towards different areas of the body depending upon which area has been affected by the cancer.

These treatments include:

Surgery: This may entail moving part or all of a lung and some of the surrounding tissue in order to remove the tumour and cancerous cells. It may also be necessary to drain the lungs of fluid, simply to make the patient more comfortable. The extent of surgery will depend largely upon the extent to which the cancer has spread.

Chemotherapy: For this procedure, drugs are used to fight the cancerous cells and kill them as well as shrink the tumour. These drugs can be swallowed by mouth if they are in tablet form, or the doctor may administer them needle directly into a vein or muscle. The drugs are then able to travel through the body via the blood stream and kill off any cancer cells that they come across.

Radiation Therapy: This was where high energy x-rays are used either outside the body (external radiation) or directed to the source internally (internal radiation) to shrink the tumour and kills off cancer cells. Internal radiation was preformed through the use of plastic tubing, where the doctor can also administer drugs.

2. Peritoneal mesothelioma

Peritoneal Mesothelioma was the name given to the cancer that attacks the lining of the abdomen. This type of cancer was caused by asbestos exposure, and it affects the lining that protects the contents of the abdomen and which also provides a lubricating fluid to enable the organs to move and work properly.

This disease was sometimes referred to as diffuse peritoneal mesothelioma, and this was where the cancer has spread out. Accountable for around ten percent of mesothelioma cases, this type asbestos-related cancer was the second most common (after Pleural Mesothelioma).

There are a number of symptoms for this disease. However, like all other types of mesothelioma, there was a very long latency period, and symptoms may not become evident for decades after exposure. This means that by the time symptoms have begun, it was often already too late to offer any real constructive treatment. Further delay can be caused due to the fact that the symptoms are generally non-specific and can therefore be attributed to a number of more common ailments. Unless your doctor was aware that you have been exposed to asbestos in the past, he or she was likely to look at other options before even contemplating mesothelioma. It was therefore important to let your doctor know of any exposure to this substance.

The symptoms of peritoneal mesothelioma include: abdominal pains and weakness; weight loss; nausea; loss off appetite; abdominal swelling; bowel obstruction. Depending on the location of the tumour, additional problems can be experienced such as breathing problems and severe pains.

Treatment for peritoneal mesothelioma can also vary, and was dependant upon a number of factors. The doctor, once a diagnosis has been made, will make a recommendation based upon details such as: the extent of the cancer and how advanced it is; the patient's general condition and health; past

medical history of the patient; and the patient's age.

Treatments include:

Surgery: This entails cutting out part of the lining and tissue from the abdomen in order to remove the tumour. Whether or not surgery was performed and how much tissue was removed will depend on how far the cancer has spread and how big the tumour is. It may sometimes be necessary to remove a lung or part of the diaphragm in order for surgery to succeed in removal of the tumour.

Radiation Therapy: This was where high energy x-rays are used to shrink tumours and kill off cancerous cells in the affected area. There are two ways to administer radiation therapy. The first was through external means, where a machine was placed outside the body and emits radiation through the body. The second was by placing a source of radiation directly to the affected area by way of plastic tubes. As well as administering the radiation treatment through the tubes, doctors are also able to administer any required drugs this way.

Chemotherapy: This was where a combination of drugs was used to try and kill off cancer cells. Drugs can be administered by mouth in tablet form, or you may have to have them administered intravenously via needle into a vein or a muscle. The drug can then enter the blood stream and travel through the body, hopefully killing off any cancer cells along the way.

Peritoneal Mesothelioma was responsible for many deaths each year. Although it was not the most common form of this cancer, many new cases come to light each year. However, with continued research and clinical trials, it may be possible to one day make this a curable disease. Peritoneal Mesothelioma was the name given to the cancer that attacks the lining of the abdomen. This type of cancer was caused by asbestos exposure, and it affects the lining that protects the contents of the abdomen and which also provides a lubricating fluid to enable the organs to move and work properly.

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3. Pericardial mesothelioma

It was the rarest form of this asbestos-related cancer. This form of cancer affects the lining that surrounds the heart, and was associated with long term exposure to asbestos fibres.

The symptoms of pericardial mesothelioma, as with other types of mesothelioma, can take decades to manifest. If a person worked with asbestos twenty or thirty years ago and shows no symptoms, that does not mean that they have the all clear. The symptoms typically take around twenty or thirty years to manifest anyway, sometimes even longer.

This means that the cancer was usually too advanced to treat effectively by the time it was diagnosed. It was always advisable that people who have worked with asbestos on a frequent basis in the past inform their doctors. Although nothing can be done to speed up the onset of symptoms in order to catch the disease in time to treat, any further delay in diagnosis can be alleviated by making your doctor aware of all the facts so that a speedy diagnosis can be made if the symptoms to manifest.

There are several main symptoms to look out for with pericardial mesothelioma. These include: persistent coughing; shortness of breath; chest pain; palpitations. Anyone that has worked with asbestos and experiences any or all of these symptoms should seek medical advice immediately.

Once your doctor has made a diagnosis, it was important to assess the extent to which the disease has spread and to what degree it has advanced. This was usually determined by imaging. A CT Scan or MRI Scan can normally reveal what stage the disease was at, and this will help to determine what sort of treatment can be considered. The patient's age, medical condition, and past medical history will also be taken into account when deciding upon a course of treatment.

Pericardial mesothelioma can occasionally be treated with surgery. However, the nature of this cancer means that it was very advanced by the time that it was diagnosed and this often means that surgery for this particular type of mesothelioma has to be ruled out. Even if surgery was performed, it was unlikely that all of the tumour or cells can be removed or treated, hence further treatment of radiation or chemotherapy (known as systemic treatments) was needed afterwards anyway.

The two most common forms of treatment for pericardial mesothelioma are radiation therapy or chemotherapy. Radiation therapy was the most commonly used of the two procedures for this type of mesothelioma, and the one that has proven most successful with pericardial mesothelioma patients to date. However, both types of treatment must be carefully monitored due to the risk they pose to healthy cells as well as affected ones.

Another low risk procedure often used simply to relieve symptoms was a 'fine needle aspiration.' This was where a needle was inserted into the chest cavity in order to drain off excess fluid build up caused by the cancer. This can often relieve some of the symptoms of the disease.

Clinical trials into all types of mesothelioma are still being continued all over the country and all over the world. Many patients find that it helps to be a part of a clinical trial as it gives them a chance to try a new form of treatment that could be effective, as well as giving them hope and making them feel more positive. And it was through this type of positive attitude along with the hard work of the professionals that, one day, a cure for all types of mesothelioma at all stages could be discovered.

3. GENOMIC ANALYSIS:

There are 44 genes have been related to Mesothelioma cancer, Out of these 41 genes were mainly responsible for Human mesothelioma remaining was for Rat mesothelioma cancer.

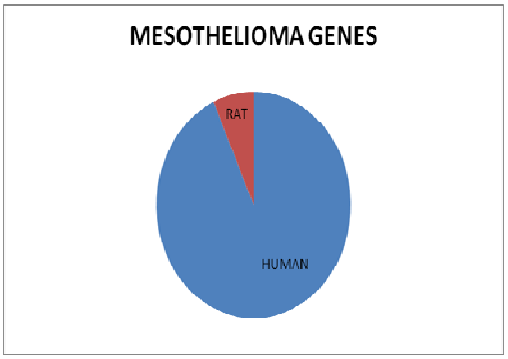

Figure 1GENES IN MESOTHELIOMAGENES IN MESOTHELIOMA

Literature evidence has proved that MSLN, NF2, TP53 are main tumor producing gene.Our research was focusing on MSLN gene hence it was a promising candidate for tumor specific therapy and it was used as a biomarker also.

MSLN gene was cell surface glycoprotein, expressed in normal epithelial cells and highly expressed in mesothelioma,ovarian cancer and adrenocarcinoma. The mesothelin gene[MSLN gene] encodes a precursor protein that was processed to yield the 40-kDa protein, mesothelin, attached to the cell membrane by a glycosylphosphatidyl inositol linkage [Anti-alopecia factor] and a 31-kDa shed fragment named megakaryocyte-potentiating factor. Mesothelin has been implicated as a potential ideal target antigen for the development of antigen-specific cancer immunotherapy for the control of mesothelin-expressing cancers such as ovarian cancer, mesothelioma and pancreatic adenocarcinoma. Mesothelin was an attractive target for cancer immunotherapy due to its restricted expression in normal tissues and high level expression in several tumors.

Three transcripts variants have been described for mesothelin gene and their protein products were named megakaryocyte potentating factor (MPF), mesothelin,& the soluble member(s) of the mesothelin MPF family. Although it was reported in 1992 that immunostaining with the K1 anti-mesothelin antibody could be very useful in distinguishing between epithelioid mesotheliomas and pulmonary adenocarcinomas .The mesothelin gene[MSLN gene] encodes a precursor protein that was processed to yield the 40-kDa protein, mesothelin attached to the cell membrane by a glycosyl phosphatidyl inositol linkage and a 31-kDa shed fragment named megakaryocyte-potentiating factor.

Mesothelin was a differentiation antigen present on the surface of ovarian cancers [Ovarian Carcinoma], mesotheliomas, and several other types of human cancer. Because among normal tissues, mesothelin was present only on mesothelial cells, it represents a good target for antibody-mediated delivery of cytotoxic agents.Mesothelin gene has limited expression on normal tissues and it highly expressed in mesothelima. The goal of the research was to find in which condition mesothelin gene was overexpresssed. Further analysis was directed on the role of MSLN gene in mesothelioma and to develop the suitable drug targets.

4. LITERATURE REVIEW

In the past 20 years, Mesothelioma was relatively common cancer

and its global incidence continues to increase.The incidence was approximately one per 1,000,000. For comparison, populations with high levels of smoking can have a lung cancer incidence of over 1,000 per 1,000,000. Incidence of malignant mesothelioma currently ranges from about 7 to 40 per 1,000,000 in industrialized Western nations, depending on the amount of asbestos exposure of the populations during the past several decades. It has been estimated that incidence may have peaked at 15 per 1,000,000 in the United States in 2004. (Bruce W. S. Robinson and Richard A 2005).

Incidence was expected to continue increasing in other parts of the world. Mesothelioma occurs more often in men than in women and risk increases with age, but this disease can appear in either men or women at any age. Approximately one fifth to one third of all mesotheliomas was peritoneal. There has been strong interest in the research for a Mesothelioma that would be of value for the genomics, proteomics and drug designing field. Males are at a much higher risk for mesothelioma than females due to occupational exposure (plumbers, pipe fitters, insulation installers, shipyard workers).

Between 1940 and 1979, approximately 27.5 million people were occupationally exposed to asbestos in the United States. Between 1973 and 1984, there has been a threefold increase in the diagnosis of pleural mesothelioma in Caucasian males. From 1980 to the late 1990s, the death rate from mesothelioma in the USA increased from 2,000 per year to 3,000, with men four times more likely to acquire it than women. These rates may not be accurate, since it was possible that many cases of mesothelioma are misdiagnosed as adenocarcinoma of the lung, which was difficult to differentiate from mesothelioma.

ASBESTOS:

Asbestos was composed of minerals, known since antiquity, with long, thin fibrous crystals. The word "asbestos" was derived from a Greek adjective meaning inextinguishable. The Greeks termed asbestos the "miracle mineral" because of its soft and pliant properties, as well as its ability to withstand heat.

Asbestos became increasingly popular among manufacturers and builders in the late 19th century due to its resistance to heat, electricity and chemical damage, sound absorption and tensile strength. When asbestos was used for its resistance to fire or heat, the fibers are often mixed with cement or woven into fabric or mats. Asbestos was used in brake shoes and gaskets for its heat resistance, and in the past was used on electric oven and hotplate wiring for its electrical insulation at elevated temperature, and in buildings for its flame-retardant and insulating properties, tensile strength, flexibility, and resistance to chemicals. Since the mid 1980s, many uses of asbestos are banned in many countries.

Working with asbestos was the major risk factor for mesothelioma. A history of asbestos exposure exists in almost all cases. However, mesothelioma has been reported in some individuals without any known exposure to asbestos. In rare cases, mesothelioma has also been associated with irradiation, intrapleural thorium dioxide (Thorotrast), and inhalation of other fibrous silicates, such as erionite.

Although asbestos has been banned in developed countries, asbestos continues to be used at an alarming rate in Southeast Asia and China. With expansion of industrialization, it was expected that within the next few decades a "mesothelioma epidemic" may be seen in this region.

Types of asbestos and associated fibers:

White asbestos:

Chrysotile, was obtained from serpentine rocks which was common throughout the world. The rocks are called serpentine because their fibers curl; chrysotile fibers are curly as opposed to fibers from amosite, crocidolite, tremolite, actinolite, and anthophyllite which are needlelike. Chrysotile, along with other types of asbestos, has been banned in dozens of countries and was only allowed in the United States and Europe in very limited circumstances. Chrysotile was used more than any other type and accounts for about 95% of the asbestos found in buildings in America. Applications where chrysotile might be used include the use of joint compound. It

was more flexible than amphibole types of asbestos; it can be spun and woven into fabric.

Brown asbestos:

Amosite, was a trade name for the amphiboles belonging to the Cummingtonite - Grunerite solid solution series, commonly from Africa, named as an acronym from Asbestos Mines of South Africa. One formula given for amosite was Fe7Si8O22(OH)2. It was found most frequently as a fire retardant in thermal insulation products and ceiling tiles.

Blue asbestos:

Crocidolite was an amphibole from Africa and Australia. It was the fibrous form of the amphibole riebeckite. One formula given for crocidolite was Na2Fe2+3Fe3+2Si8O22(OH)2.Notes:chrysotile commonly occurs as soft friable fibers. Asbestiform amphibole may also occur as soft friable fibers but some varieties such as amosite are commonly straighter. All forms of asbestos are fibrillar in that they are composed of fibers with widths less than 1 micrometre that occur in bundles and have very long lengths. Asbestos with particularly fine fibers was also referred to as "amianthus". Amphiboles such as tremolite have a crystal structure containing strongly bonded ribbonlike silicate anion polymers that extend the length of the crystal. Serpentine (chrysotile) has a sheetlike silicate anion which was curved and which rolls up like a carpet to form the fiber.

Other asbestos:

Other regulated asbestos minerals, such as tremolite asbestos, CAS No. 77536-68-6, Ca2Mg5Si8O22(OH)2; actinolite asbestos (or smaragdite), CAS No. 77536-66-4, Ca2(Mg, Fe)5(Si8O22)(OH)2; and anthophyllite asbestos, CAS No. 77536-67-5, (Mg, Fe)7Si8O22(OH)2; are less commonly used industrially but can still be found in a variety of construction materials and insulation materials and have been reported in the past to occur in a few consumer products.

Other natural and not currently regulated asbestiform minerals, such as richterite, Na(CaNa)(Mg,Fe++)5(Si8O22)(OH)2, and winchite, (CaNa)Mg4(Al,Fe3+)(Si8O22)(OH)2, may be found as a contaminant in products such as the vermiculite containing zonolite insulation manufactured by W.R. Grace and Company. These minerals are thought to be no less harmful than tremolite, amosite, or crocidolite, but since they are not regulated, they are referred to as "asbestiform" rather than asbestos although may still be related to diseases and hazardous.

Specific products:

Serpentine group

Serpentine minerals have a sheet or layered structure. Chrysolite was the only asbestos mineral in the serpentine group. In the United States, chrysotile has been the most commonly used type of asbestos. According to the U.S. EPA Asbestos Building Inspectors Manual, chrysotile accounts for approximately 95% of asbestos found in buildings in the United States. Chrysotile was often present in a wide variety of materials, including:

- joint compound
- mud and texture coats
- vinyl floor tiles, sheeting, adhesives
- roofing tars, felts, siding, and shingles
- "transite" panels, siding, countertops, and pipes
- fireproofing
- chaulk
- gaskets
- brake pads and shoes
- clutch plates
- stage curtains
- fire blankets
- interior fire doors
- fireproof clothing for firefighters
- thermal pipe insulation

Amphibole group:

Five types of asbestos are found in the amphibole group: amosite, crocidolite, anthophyllite, tremolite, and actinolite. Amosite, the second most likely type to be found in buildings, according to the U.S. EPA Asbestos Building Inspectors Guide, was the "brown" asbestos.

Amosite and crocidolite were formerly used in many products until the early 1980s. The use of all types of asbestos in the amphibole group was banned (in much of the Western world) by the mid-1980s and by Japan in 1995. These products were mainly:

- Low density insulation board and ceiling tiles
- Asbestos-cement sheets and pipes for construction, casing for water and electrical/telecommunication services
- Thermal and chemical insulation (i.e., fire rated doors, limpet spray, lagging and gaskets)

Other asbestos-related diseases:

- Asbestos warts – caused when the sharp fibers lodge in the skin and are overgrown causing benign callus-like growths.

- Pleural plaques – discrete fibrous or partially calcified thickened area which can be seen on X-rays of individuals exposed to asbestos. They do not become malignant or cause other lung impairment.

- Diffuse pleural thickening – similar to above and can sometimes be associated with asbestosis. Usually no symptoms shown but if extensive can cause lung impairment.

Contamination of other products:

(a) Asbestos and vermiculite:

Vermiculite was a hydrated laminar magnesium-aluminum-iron silicate which resembles mica. It can be used for many industrial applications and has been used as a replacement for asbestos. Some ore bodies of vermiculite have been found to contain small amounts of asbestos. One vermiculite mine operated by W. R. Grace and Company in Libby, Montana exposed workers and community residents to danger by mining contaminated vermiculite, in 1999 the EPA began cleanup efforts and now the area was a superfund cleanup area. The EPA has determined that harmful asbestos was released not only from the mine, but also through other activities that disturb soil in the area.

(b) Asbestos and talc:

Talc was sometimes contaminated with asbestos. In 2000, tests in a certified asbestos-testing laboratory found the tremolite form of amphibole asbestos in three out of eight major brands of children's crayons (oil pastels) that are made partly from talc — Crayola, Prang, and Rose Art. In Crayola crayons, the tests found asbestos levels from 0.05% in Carnation Pink to 2.86% in Orchid; in Prang crayons, the range was from 0.3% in Periwinkle to 0.54% in Yellow; in Rose Art crayons, it was from 0.03% in Brown to 1.20% in Orange. Overall, 32 different types of crayons from these brands contained more than trace amounts of asbestos, and eight others contained trace amounts. The Art and Creative Materials Institute, a trade association which tests the safety of crayons on behalf of the makers, initially insisted the test results must be incorrect, although they later said they do not test for asbestos. In May 2000, Crayola said tests by materials analyst, Richard Lee, of two of its crayons were negative for asbestos, although it later emerged that Lee had testified in lawsuits over 250 times on behalf of the asbestos industry, which paid him US$7 million. In June 2000, Binney & Smith, the maker of Crayola, and the other makers agreed to stop using talc in their products, and changed their product formulations in the United States. The mining company, R T Vanderbilt Co of Gouvernor, New York, which supplied the talc to the crayon makers, insists there was no asbestos in its talc "to the best of our knowledge and belief", but tests by the United States Mine Safety and Health Administration found asbestos in all four talc samples that it tested in 2000.

Asbestos construction in developing countries:

Some developing countries, such as India and China, have continued widespread use of asbestos. The most common was corrugated asbestos-cement sheets or "A/C Sheets" for roofing and for side walls. Millions of homes, factories, schools or sheds and shelters continue to use asbestos. Everest Industries (formerly

Eternit Everest), Hyderabad, Visakha Industries and RamCo are some of the major asbestos products manufacturers in India.

Cutting these sheets to size and drilling holes to receive 'J' bolts to help secure the sheets to roof framing was done on site. There has been no significant change in production and use of A/C Sheets in developing countries following the widespread restrictions in developed nations.

5. MATERIALS & METHODS

Literature review has proven that mesothelin was a gene which was mainly responsible for asbestos induced mesothelioma cancer. Our further analysis was focused on the mesothelin gene using bioinformatics techniques. Genomic studies on Mesothelin gene leads to discovery of the newer drug target and therapeutics

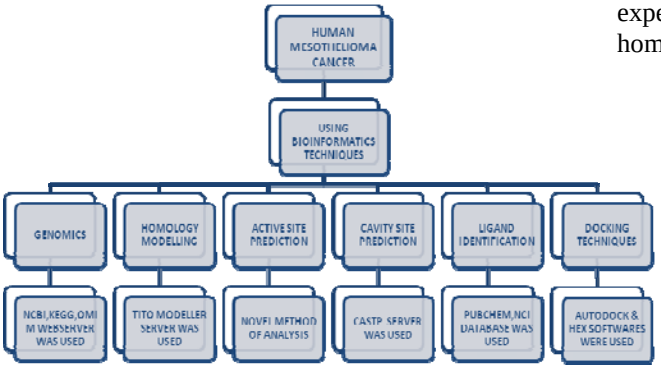

Figure 2 *Methodology for analysis of mesothelin gene*

In protein structure prediction, homology modeling, also known as comparative modeling, was a class of methods for constructing an atomic-resolution model of a protein from its amino acid sequence (the "query sequence" or "target"). Almost all homology modeling techniques rely on the identification of one or more known protein structures (known as "templates" or "parent structures") likely to resemble the structure of the query sequence, and on the production of an alignment that maps residues in the query sequence to residues in the template sequence. The sequence alignment and template structure are then used to produce a structural model of the target. Because protein structures are more conserved than protein sequences, detectable levels of sequence similarity usually imply significant structural similarity.

The method of homology modeling was based on the observation that protein tertiary structure was better conserved than amino acid sequence. Thus, even proteins that have diverged appreciably in sequence but still share detectable similarity will also share common structural properties, particularly the overall fold. Because it was difficult and time-consuming to obtain experimental structures from methods such as X-ray crystallography and protein NMR for every protein of interest, homology modeling can provide useful structural models for generating hypotheses about a protein's function and directing further experimental work. The steps involved in the homology modelling are as follows.

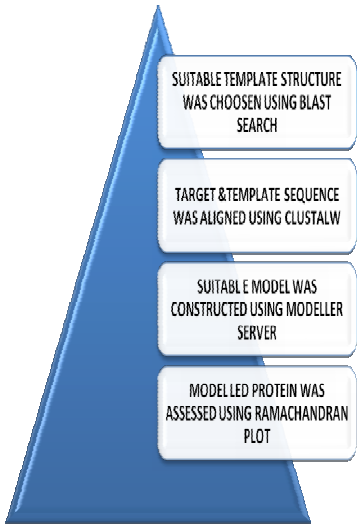

FIGURE 3 METHODOLOGY FOR HOMOLOGY MODELING

IDENTIFICATION OF ACTIVESITE:

Novel method of identification of the active site was analyzed by insilico comparison of the two isoform variants of the mesothelin gene. It hints that some of the amino acids were missing in the isoform2. Mesothelin family of proteins, found that patients with malignant mesothelioma had a higher levels of serum mesothelin related peptide (SMR) than the healthy controls and it gave the clue for the further analysis of mesothelin gene.

CAVITY SITE PREDICTION:

The suitable cavity site was predicted by using CASTp server (Computed Atlas Of Surface Topography Of Protein). Binding sites and active sites of proteins and DNAs are often associated with structural pockets and cavities. castP server uses the weighted Delaunay triangulation and the alpha complex for shape measurements. It provides identification and measurements of surface accessible pockets as well as interior inaccessible cavities, for proteins and other molecules. It measures analytically the area and volume of each pocket and cavity, both in solvent accessible surface (SA, Richards' surface) and molecular surface (MS, Connolly's surface). It also measures the number of mouth openings, area of the openings, and circumference of mouth lips, in both SA and MS surfaces foreachpocket. Sought to request a calculation for a particular molecule. The results will be shown on the screen or emailed to us. The emailed results include measured parameters for pockets, cavities and mouth openings, as well as listing of wall atoms and mouth atoms for each pocket. In addition, a downloadable PyMOL plugin will help us to visualize the pocket of our interest. In CASTp calculation request, the modeled protein was submitted and the suitable cavity selected.

LIGAND IDENTIFICATION:

Once the therapeutic target has been identified, we must then find one or more leads (e.g., chemical compounds or molecules) that interact with the therapeutic target so as to induce the desired therapeutic effect, e.g., through antiviral or antibacterial activity. In order to discover the compounds whose pharmacological properties are likely to have the required therapeutic effects, researchers must test a large variety of them on one or more targets. The pharmaceutical companies possess veritable libraries of synthetic or natural compounds, ready to be tested. To test the chosen compounds in large numbers, scientists use an entirely automated process known as high density screening. In general, of the thousands of compounds tested, barely 1% will qualify for further and more probing analysis.

There are two ways to find Lead compounds that interact with our Target protein.

- Using Literature Survey
- Comparative analysis, finding ligands that binds to template protein and use that ligand and its similar ligands.

LEAD OPTIMIZATION:

Lead optimization was followed after lead identification whereas SBDD techniques are especially effective in refining their 3D structures to improve binding to protein active sites. In lead optimization researchers systematically modify the structure of the lead compound, docking each specific configuration of a drug compound in a protein's active site, and then testing how well each configuration binds to the site A few examples of bioinformatics tools that aid in lead optimization efforts are BIOSTER, WABE, and ClassPharmer Suite. The objective of this drug discovery phase was to optimize lead compounds i.e. new analogs with improved potency, reduced off-target activities, and physiochemical/metabolic properties suggestive of reasonable in vivo pharmacokinetics. Mol inspiration helps in optimizing lead by calculating molecular physicochemical properties relevant to drug design and QSAR, including logP, molecular polar surface area (PSA), and the Rule of 5 descriptors. Lipinski's Rule of Five was a rule of thumb important for drug development where a pharmacologically active lead structure was optimized step-wise for increased activity and selectivity. The modification of the molecular structure often leads to drugs with higher molecular weight, more rings, more rotatable bonds, and a higher lipophilicity.

Lipinski's Rule of Five states that, in general, an orally active drug has:

- Not more than 5 hydrogen bond donors (OH and NH groups)
- Not more than 10 hydrogen bond acceptors (notably N and O)
- A molecular weight under 500 g/mol
- A partition coefficient log P less than 5

DOCKING TECHNIQUES:

To predict the affinity, activity, binding orientation of our ligand to our target protein MESOTHELIN i.e. to perform docking using AUTODOCK.

Docking was a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. Knowledge of the preferred orientation in turn may be used to predict the strength of association or binding affinity between two molecules using for example scoring functions. The associations between biologically relevant molecules such as proteins, nucleic acids, carbohydrates, and lipids play a central role in signal transduction. Furthermore, the relative orientation of the two interacting partners may affect the type of signal produced (e.g., agonism vs. antagonism). Therefore docking was useful for predicting both the strength and type of signal produced.

Docking was frequently used to predict the binding orientation of small molecule drug candidates to their protein targets in order to in turn predict the affinity and activity of the small molecule. Hence docking plays an important role in the rational design of drugs. Given the biological and pharmaceutical significance of molecular docking, considerable efforts have been directed towards improving the methods used to predict docking.

AutoDock was a suite of automated docking tools. It was designed to predict how small molecules, such as substrates or drug candidates, bind to a receptor of known 3D structure. AutoDock actually consists of two main programs: AutoDock performs the docking of the ligand to a set of grids describing the target protein; Auto Grid pre-calculates these grids. In addition to using them for docking, the atomic affinity grids can be visualized. This can help, for example, to guide organic synthetic chemists design better binders. Also there was a graphical user interface called AutoDockTools, or ADT for short, which amongst other things helps to set up which bonds will treated as rotatable in the ligand and to analyze dockings.

AutoDock has applications in:

- X-ray crystallography;
- structure-based drug design;
- lead optimization;
- virtual screening (HTS);
- combinatorial library design;
- protein-protein docking;
- chemical mechanism studies

Software

AutoDock : http://autodock.scripps.edu/

Procedure

Steps involved in AutoDock

Initially save the protein and the ligand molecule as protein.pdb and ligand.pdb in a new folder in the desktop. Here we do AutoDock4 in Linux.

- Open terminal and give in the following commands
  - cd Desktop
  - cd New Folder
  - ls
  - adt
- Prepare protein molecule

- File → Read Molecule → Open (protein.pdb).
- Edit → Hydrogens → Add → Polar Only.
- Select → Select from string → (Type * for Molecule, chain and atom and HOH for Residue) Click add → If water present go to edit and delete it from delete atom set, If absent click dismiss.

- Save → Write PDB → (Add ATOM, CONNECT, TER, END and select sort nodes and save transformed coordinates) OK.

➢ Prepare Ligand molecule

- Ligand → Input → Open (ligand. pdb)
- Press N to make ligand visible.
- Ligand → Torsion tree → Detect root → Root Expansions
- Ligand → Choose torsions.Ligand → Set NO of torsions
- Ligand → Output → Save as PDBQT (ligand. pdbqt)

➢ Prepare Flexible and Rigid file

- Flexible Residues → Input → Choose Macromolecule → Select Protein
- (To select the active site) Select → Select from string → (Type * for Molecule, chain and atom and Active site residue for Residue) Click add.
- Flexible Residues → Choose torsions in residues → close.
- Flexible Residues → Output → Save flexible pdbqt (flex.pdbqt)
- Flexible residues → Redisplay macromolecule.
- Flexible residues → Output → Save Rigid pdbqt (rigid.pdbqt)

➢ Set Grid Box

- Grid → Macromolecule → Choose → Protein
- Save as protein.pdbqt.
- Grid → Set Map Types → Directly → Accept.
- Grid → Grid Box (Select the three coordinates such that the active site was covered in the Grid map. Note that three coordinates should be equal), Click centre and then Pick an atom to place our active site as centre of grid. Go to file in grid box then Close saving Current.
- Grid → Output → Save(protein.gpf)
- (To display label of active site) Display → label → by properties → label only → residue → name.

➢ Running the Grid

- Run → Run Autogrid(give in the program path for the autogrid file and the .gpf file)

Wait until the Successful completion of Autogrid was displayed in the terminal.

➢ Docking

- Docking → Macromolecule → Choose rigid molecule → rigid.pdbqt.
- Docking → Ligand → Choose → Select ligand → Accept.
- Docking → Output → Lamarckian GA → Save dpf (protein.pdb)

➢ Run Docking

- Run → Run AutoDock (Give in the pathname for AutoDock and Dpf file).

Wait until successful completion of Docking.

➢ Visualize the Docking Results

- Analyze → Docking → Open → Protein.dlg → ok
- Analyze → Macromolecule → Choose → protein
- Analyze → conformations → Play
- Color by molecule → Show information → build H atoms

MOLECULAR DOCKING USING HEX:

Hex was an interactive protein docking and molecular superposition program. Currently, Hex understands protein and DNA structures in PDB format. Hex was written by Dave Ritchie at the University of Aberdeen.

The main thing which distinguishes Hex from other macromolecular (i.e. protein and DNA) docking programs and molecular graphics packages was its use of polar Fourier correlations to accelerate docking

calculations. The graphical nature of Hex came about largely because I wanted to visualise the results of such docking calculations in a natural and seamless way, without having to export unmanageably many (and usually quite big) coordinate files to one of the many existing molecular graphics packages. For this reason, the graphical capabilities in Hex are relatively primitive, although these days one can do quite a lot with a few calls to OpenGL.

In Hex's docking calculations, each molecule was modelled using 3D parametric functions which are used to encode both surface shape and electrostatic charge and potential distributions. The parametric functions are based on expansions of real orthogonal spherical polar basis functions. Essentially, this allow each property to be represented by a vector of coefficients. Hex's surface shape representation uses a novel 3D surface skin model of protein topology, whereas the electrostatic model was derived from classical electrostatic theory. By writing an expression for the overlap of pairs of parametric functions, one can derive an expression for a docking score as a function of the six degrees of freedom in a rigid body docking search. With suitable scaling factors, this docking score can be interpreted as an interaction energy, which we seek to minimise. Due to the special orthogonality property of the basis functions, the correlation (or overlap as a function of translation/rotation operations) between a pair of 3D functions can be calculated using expressions which involve only the original expansion coefficients. In many respects, this approach was similar to conventional fast Fourier transform (FFT) docking methods which use a Cartesian grid to perform the Fourier transforms. However, the FFT approach only accelerates a docking search in three (translational) degrees of freedom whereas with a spherical polar approach, we can both translate (with some effort) and rotate (relatively easily) the coefficient vectors to generate and evaluate candidate docking orientations in what was effectively a six dimensional Fourier correlation.

In the spherical polar approach, it was natural to assign the six rigid body degrees of freedom as five Euler rotation angles and an intermolecular separation. Thus, in complete contrast to the FFT approach, the rotational part of a docking search was the "easy bit" and modelling translations becomes the "hard part". Fortunately, however, only a few translations (typically about 40 steps of 0.75 Ångstrom) are required to complete a six dimensional docking search. A further advantage of the spherical polar approach was that it was easy to constrain the docking search to one or both binding sites, when this knowledge was available, simply by constraining one or two of the angular degrees of freedom. This can reduce docking times to a matter of minutes on modern workstations. So, depending on how well a particular FFT algorithm was implemented (and on who you believe!), I claim that Hex was somewhere between 10 and 100 times faster than conventional FFT docking algorithms.

Closely related to the protein docking problem was the molecular similarity problem - i.e. how to find the relative orientation of a pair of similar molecules such that some measure of the similarity (difference) between the molecules was maximised (minimised). Both problems involve translating and rotating one or both molecules into the desired orientation. However, to a first approximation, the similarity problem can be reduced to a three dimensional rotational search by initially placing both molecules in a common coordinate system.

In fact, much of the early development of Hex concentrated on displaying and superposing protein surface shapes using two dimensional spherical harmonic expansions to represent surface shapes parametrically. This proved to be a fast and accurate way to superpose pairs of similar protein molecules but this type of 2D surface approach does not encode sufficient detail to give a viable docking algorithm. It was this observation that prompted the development of our 3D density model of molecular shape.

STEPS INVOLVED:

1. To run a docking calculation in Hex, need to load a receptor and a ligand PDB structure using the File pull-down menu.
2. To test the docking algorithm by docking two separately determined sub-units of a complex for which the

crystal structure was also available, also load the complex structure which will be used as a reference orientation to evaluate the accuracy of the docking prediction.

3. It was need to remove water molecules and any other hetero molecules prior to docking. Alternative method was globally using the Hetero Control menu panel.
4. If more detailed control was required, probably have to edit each PDB file manually.
5. It may also be necessary to remove other chains in the PDB file or to shorten a chain to the domain of interest in docking. For example, when docking an antigen to an antibody it was usually advisable to delete all but the Fv fragment of the antibody structure (although the program has been used to dock a protein G molecule to a complete Fab fragment).
6. Having edited the PDB files, it should have a receptor and a ligand file which contain only the receptor and ligand molecules, respectively, and (optionally) a complex file, which contains both molecules in the docked orientation.
7. When using a complex structure, you should ensure that the chain names are consistent with those of the receptor and ligand because Hex uses the chain labels to identify and hence superpose corresponding pairs of alpha-carbon atoms from each chain in order to calculate RMS deviations between the docked position of the ligand and its position in the known complex.

6. RESULTS&DISCUSSION

The genes involved in mesothelioma cancer were evaluated by using NCBI database. The number of genes involved was listed below

Target for Mesothelioma can be identified using literature review. After reviewing the Literature, a novel target was identified and Information like name and origin, entry information, references, cross references, databases and sequence of this target was retrieved in UniProtKB/TrEMBL, a computer-annotated supplement of Swiss-Prot that contains all the translations of EMBL nucleotide sequence entries not yet integrated in Swiss-Prot.

Name and origin of the protein	
Protein name	**Mesothelin [Precursor]**
Synonyms	**Pre-pro-megakaryocyte-potentiating factor** **CAK1 antigen**
Contains	**Megakaryocyte-potentiating factor** (MPF) **Mesothelin, cleaved form**
Gene name	**Name: MSLN** Synonyms: MPF
From	Homo sapiens (Human) [TaxID: 9606]
Taxonomy	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini; Catarrhini; Hominidae; Homo.
Protein existence	1: Evidence at protein level;

Figure 4 SWISSPROT ENTRY

HOMOLOGY MODELLING OF MESOTHELIN GENE:

The 3D structure of the mesothelin gene was modeled by using TITO modeller server.

TEMPLATE SELECTION The critical first step in homology modeling was the identification of the best template structure, if indeed any are available. The simplest method of template identification relies on serial pairwise sequence alignments aided by database search techniques such as FASTA and BLAST.

BLAST RESULT:

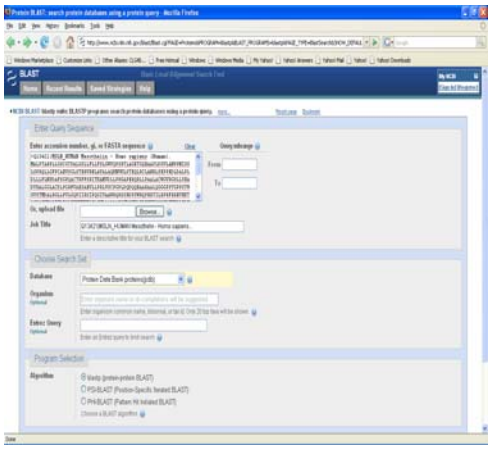

FIGURE 5 BLAST HOMEPAGE

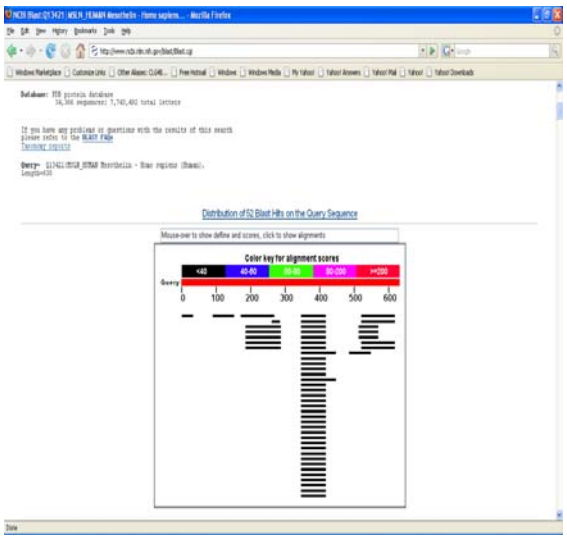

Figure 6 BLAST ALIGNMENT SCORE

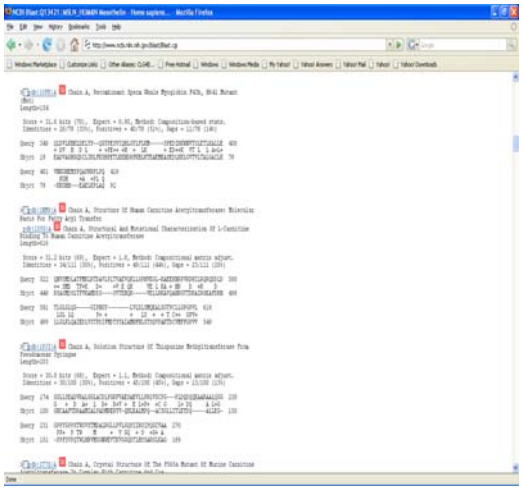

Figure 7 TEMPLATE SELECTION

The suitable template structure choosen was 10FK and it was a recombinant sperm whale myoglobulin.

TARGET-TEMPLATE SEQUENCE ALIGNMENT:

It was possible to use the sequence alignment generated by the database search technique as the basis for the subsequent model production; however, more sophisticated approaches have also been explored. One proposal generates an ensemble of stochastically defined Multiple sequence alignments (CLUSTALW) between the target sequence and a single identified template as a means of exploring "alignment space" in regions of sequence with low local similarity.

CLUSTALW RESULT:

Figure 8 CLUSTALW HOMEPAGE

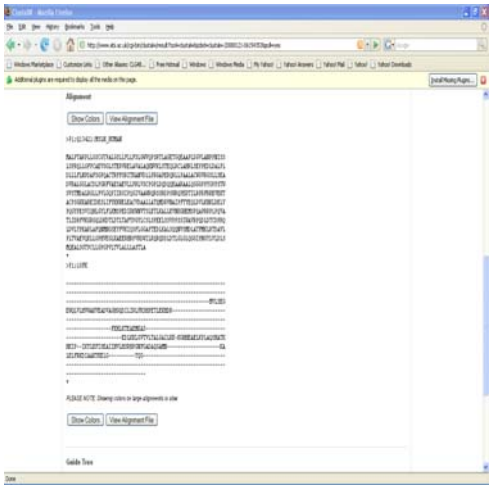

Figure 9 CLUSTALW ALIGNMENT

Model generation

Given a template and an alignment, the information contained therein must be used to generate a three-dimensional structural model of the target, represented as a set of Cartesian coordinates for each atom in the protein. Three

major classes of model generation methods have been proposed.

Tools used to model the structure:

TITO modeller server:

Paste the aligned sequences obtained from CLUSTALW of both template and target sequence in to the appropriate box.

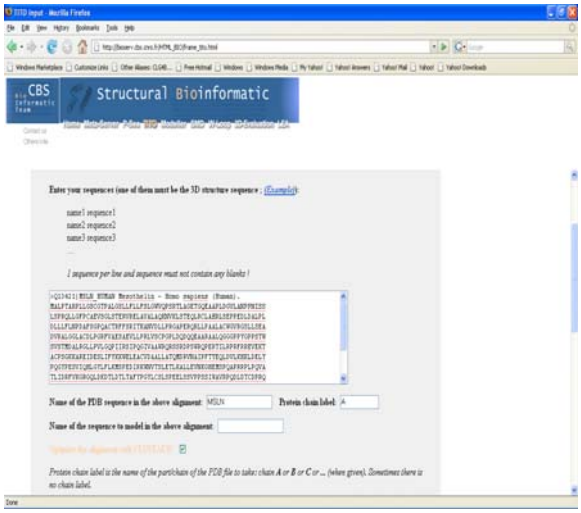

Figure 10 TITO MODELLER RESULT

STRUCTURE OF MSLN GENE:

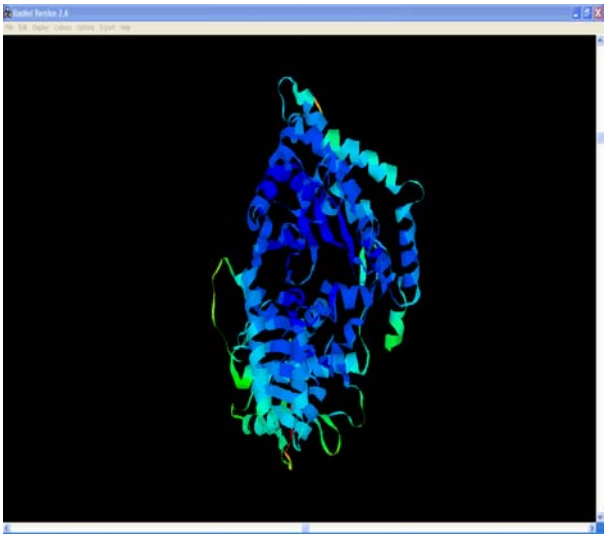

Figure 11 STRUCTURE OF MSLN

The modeled structure of the protein was also validated using the SAVS (Structural Analysis and Validation Server). It utilizes 5 programs to do the validation:

PROCHECK

WHAT_CHECK

ERRAT

VERIFY_3D

PROVE

The following results were obtained from the respective programs:

Procheck:

This tool gives details about the Ramachandran plot

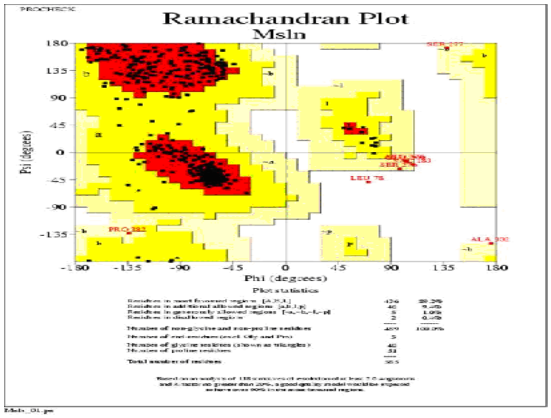

Figure 12 RAMACHANDRAN PLOT

The Modeled Structure of Novel Protein using PROCHECK program gives RAMACHANDRAN PLOT

What check:

It gives the detailed information about the analysis of the protein structure.

Errat:

The plot gives the details about the error values of each residue.

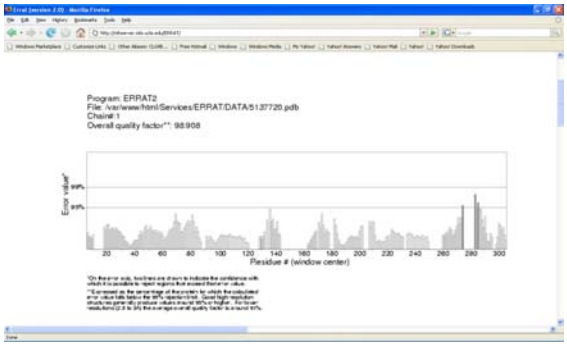

Figure 13 Modeled structure of protein residue

With the program giving the overall quality factor of the modeled structure as 98.908.

ACTIVE SITE IDENTIFICATION:

The two isoform variants of mesothelin gene was shown that some of the aminoacids were missing in isoform2.

Isoform1:

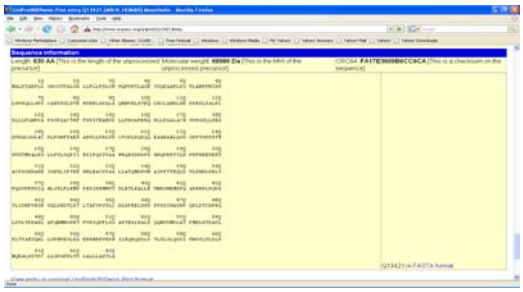

Figure 14 ISOFORM1 OF MSLN

Isoform2:

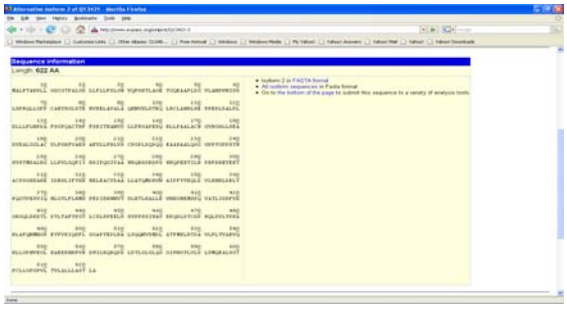

Figure 15 ISOFORM2 OF MSLN

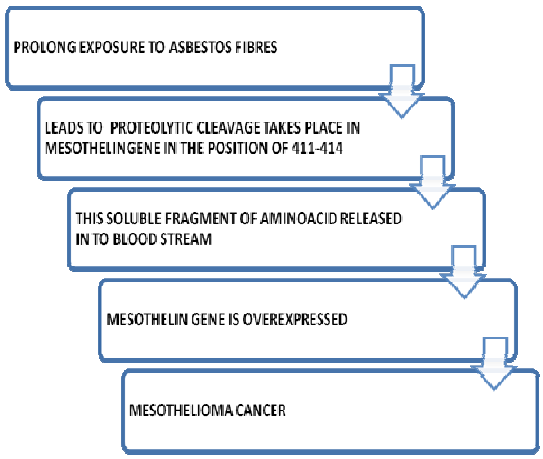

Figure 16 Novel method of identification of Active site

Our novel findings using bioinformatics approach clearly indicated that proteolytic cleavage takes place in the position of 408-414 along with PXXP motif, which causes the release of the soluble fragment of peptides into the blood stream, this was the condition in which mesothelin was over expressed. The active site identified was (408-414) along with PXXP motif,a suitable ligand should be selected to block the release of the peptides in to blood stream . Inturns it prevent the proteolysis of motif, thus reduce the expression of the mesothelin gene in the body.

CAVITY SITE PREDICTION USING CASTP:

The protein cavity was identified by using CASTp (Computed Atlas of Surface Topography of Protein)

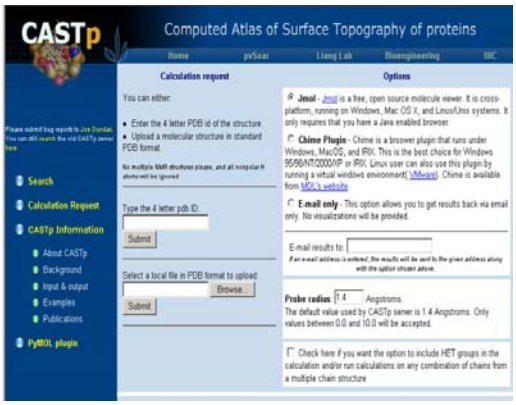

Figure 17 CASTP HOME PAGE

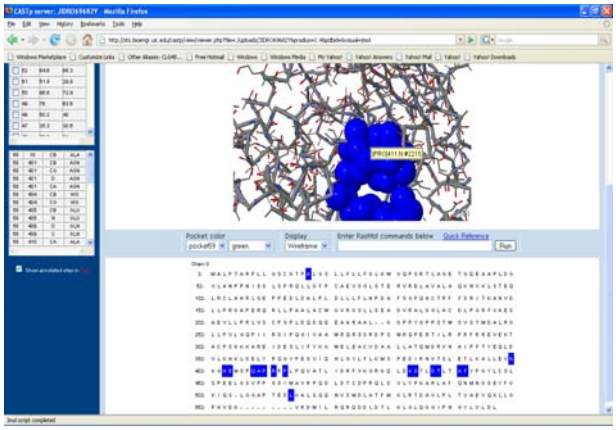

Figure 18 CAVITY SITE OF MSLN GENE

In CASTp calculation request, the modeled protein was submitted and the suitable cavity selected was PRO408,ARG409,SER410,PRO411, ARG412, ARG413, PRO414

LIGAND IDENTIFICATION:

Our research was attempted to focus on the suitable ligand such as Cyclophosphamide, Gemcitabine, Letrozole, and Mechlrothamine & Carmustine. These preferred drugs should have the activity to prevent the loss of amino acid from the mesothelin gene when it over expressed. The comparative studies of these drugs as related as follows Cyclophosphamide also known as cytophosphane was a nitrogen mustard alkylating agent, from the oxazophorines group. It was used to treat various types of cancer especially lung cancer, Breast cancer and also some autoimmune disorders. It was a "prodrug"; it was converted in the liver to active forms that have chemotherapeutic activity. Other chemotherapeutic agent gemcitabine was a nucleoside analog in which the hydrogens on the 2' carbons of deoxycytidine are replaced by fluorines.

As with fluorouracil and other analogues of pyrimidines, the drug replaces one of the building blocks of nucleic acids, in this case cytidine, during DNA replication. The process arrests tumor growth, as new nucleosides cannot be attached to the "faulty" nucleoside, resulting in apoptosis (cellular "suicide").Gemcitabine was used in various carcinomas: non-small cell lung cancer, pancreatic cancer, bladder cancer and breast cancer. It was being investigated for use in oesophageal cancer, and was used experimentally in lymphomas and various other tumor types. It was also not as debilitating as other forms of chemotherapy

Mechlorothamine was an alkylating agents and it was used as an antineoplastic in Hodgkin's disease and lymphomas. It causes severe gastrointestinal and bone marrow damage. A vesicant and necrotizing irritant destructive to mucous membranes. It was formerly used as a war gas. Other chemotherapeutic drug such as Letrozole was an oral non-steroidal aromatase inhibitor that has been introduced for the adjuvant treatment of hormonally-responsive breast cancer and also ovarian cancer. It was a successful drug candidate for ovarian cancer and breast cancer. Carmustine was a chemotherapy drug that was given as a treatment for some types of cancer. It was most commonly used to treat myeloma , ovarian cancer and brain tumours.

After a thorough literature survey, it was attempted to focusing the drugs such as Cyclophosphamide, Gemcitabine Mechlrothamine were used against most of the Lung cancer. Letrozole, Carmustine were mainly used against Ovarian Cancer. So a pubchem compound search was done for all these drugs and comparative studies were also done by using docking techniques.

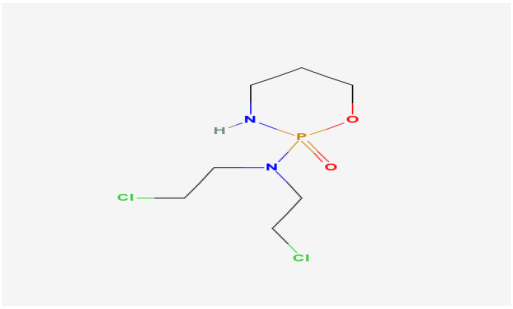

Figure 19 CYCLOPHOSPHAMIDE

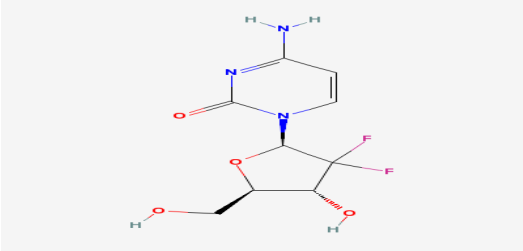

Figure 20 GEMCITABINE

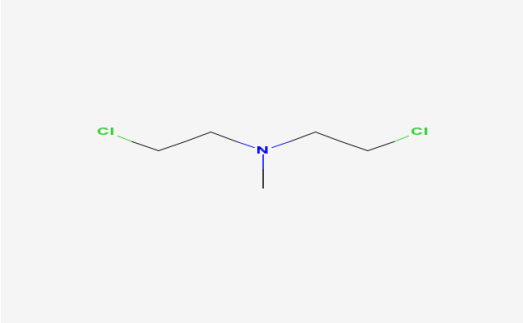

Figure 21 MECHLOROTHAMINE

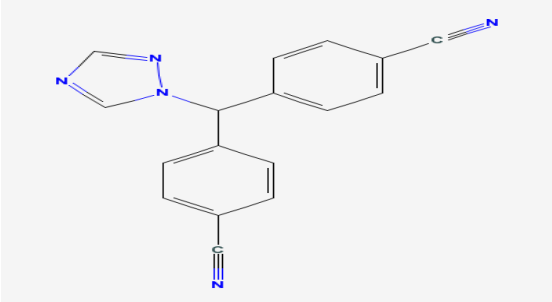

Figure 22 LETRAZOLE

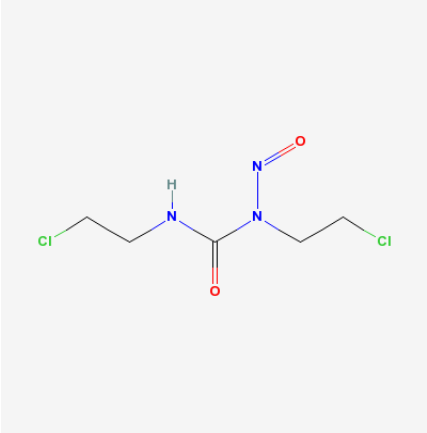

Figure 23 CARMUSTINE

LEAD OPTIMIZATION

Identified lead can be optimized by any of these three methods.

1. Lipinski Rule
2. Mol inspiration
3. PreADMET

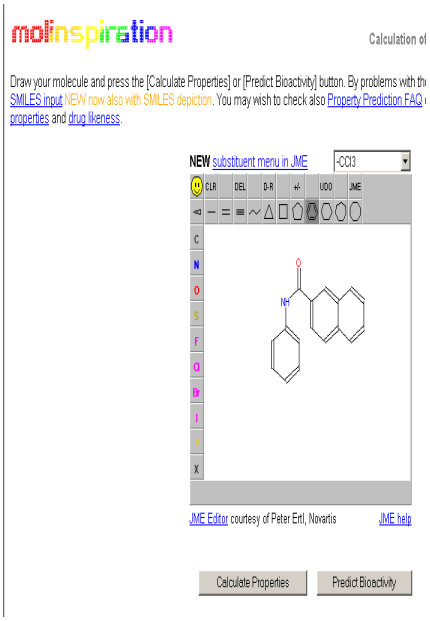

Figure 24 MOLINSPIRATION

Figure 25 PREADMET

Five leads identified in the previous step are

1. Cyclophosphamide
2. Gemcitabine
3. Mechlorothamine
4. Letrozole
5. Carmustine

Property	Numerical value
Molecular Weight	261.085961 g/mol
Molecular Formula	C7H15Cl2N2O2P
Hydrogen Bond Donor Count	1
Hydrogen Bond Acceptor Count	4
XlogP	0.8

Table 1 PROPERTIES OF CYCLOPHOSPHAMIDE

Property	Numerical value
Molecular Weight	263.198146 g/mol
Molecular Formula	C9H11F2N3O4
Hydrogen Bond Donor Count	3
Hydrogen Bond Acceptor Count	7
XlogP	-1.4

Table 2 PROPERTIES OF GEMCITABINE

Property	Numerical value
Molecular Weight	156.05354 g/mol
Molecular Formula	C5H11Cl2N
Hydrogen Bond Donor Count	0
Hydrogen Bond Acceptor Count	1
XlogP	1.6

Table 3 PROPERTIES OF MECHLOROTHAMINE

Property	Numerical value
Molecular Weight	285.30274 g/mol
Molecular Formula	C17H11N5
Hydrogen Bond Donor Count	0
Hydrogen Bond Acceptor Count	5
XlogP	2.5

Table 4 PROPERTIES OF LETRAZOLE

Property	Numerical value
Molecular Weight	214.04986 g/mol
Molecular Formula	C5H9Cl2N3O2
Hydrogen Bond Donor Count	1
Hydrogen Bond Acceptor Count	3
XlogP	1.5

Table 5 PROPERTIES OF CARMUSTINE

All these drugs have satisfied the Lipinski rule of Five, and properties of these compounds as listed as above. Hence these leads was chosen as the optimized ligand with which docking of our target protein mesothelin was to be carried out in the future steps

DOCKING RESULTS:

The lead cyclophosphamide was used to dock our target protein mesothelin and the binding energy was found to be -7.97.

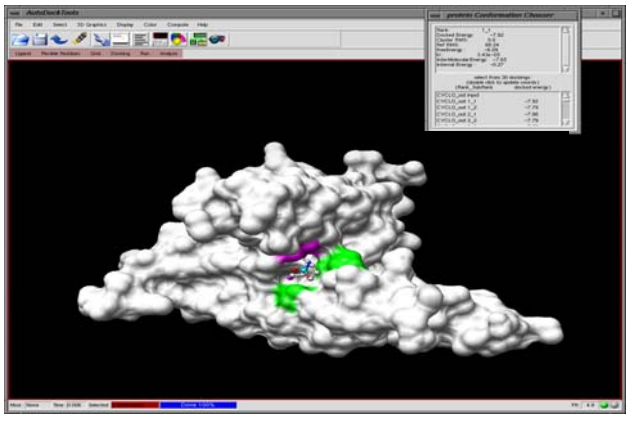

Figure 26 CYCLOPHOSPHAMIDE WAS DOCKED WITH MSLN USING AUTODOCK

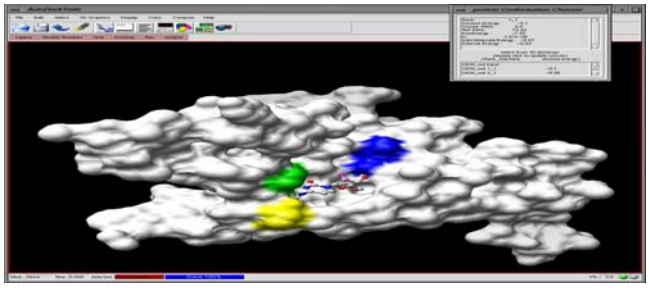

Figure 27 GEMCITABINE WAS DOCKED WITH MSLN USING AUTODOCK

The lead Gemcitabine was used to dock our target protein mesothelin and binding energy was found to be -8.10.

Mechlorothamine:

The energy results of Mechlorothamine was found to be -6.00

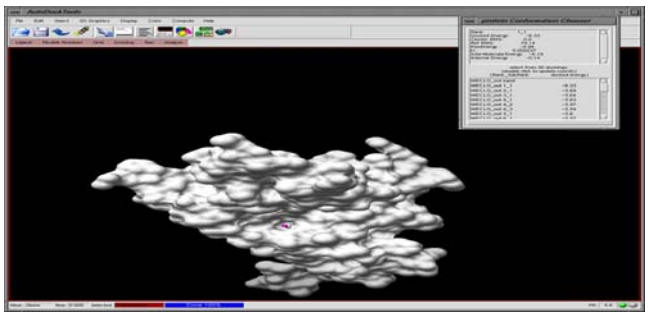

Figure 28 MECHLOROTHAMINE WAS DOCKED WITH MSLN USING AUTODOCK

Le

The minimum energy was found to be -9.49 and it showing good conformation with that of our target protein.

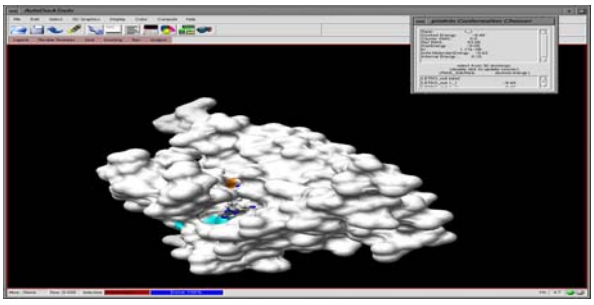

Figure 29 LETRAZOLE WITH MSLN USING AUTODOCK

CARMUSTINE The minimum energy was found to be -8.0 and it showing good conformation with that of our target protein

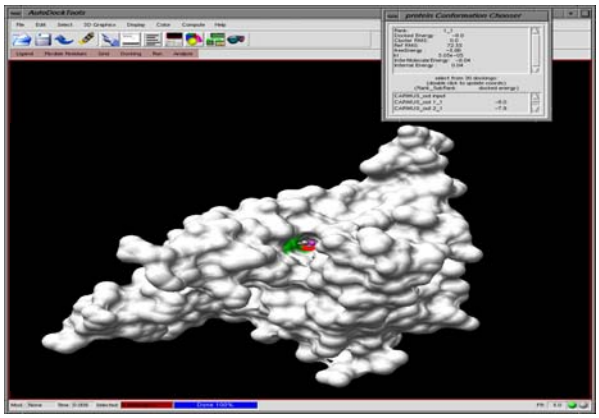

Figure 30 CARMUSTINE WITH MSLN USING AUTODOCK

On the basis of the highest docking score letrazole was found to be the good target for mesothelioma disease. Previous literature review has indicated that mesothelin gene was not only responsible for mesothelioma and it was also involved in ovarian carcinoma. Letrazole was a successful drug candidate for Ovarian cancer targeting EGFR gene. Premetrexed combination with cisplatin was usually used for mesothelioma cancer hence our research has proved that drugs used for ovarian carcinoma and lung carcinoma as ability to dock our target protein mesothelin for mesothelioma cancer.

HEX RESULTS: The HEX docking steps were performed using our ligand Cyclophosphamide to our target protein, Mesothelin – Mesothelioma cancer. The Evalue of our target protein Mesothelin was docked with cyclophosphamide was found to be -78.33.

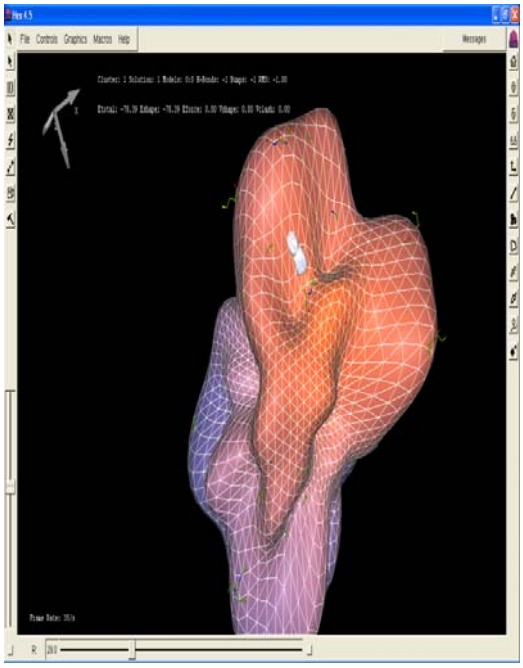

Figure 31 CYCLOPHOSPHAMIDE WAS DOCKED WITH MSLN USING HEX

Gemcitabine

The Evalue of our target mesothelin was docked with gemcitabine was found to be – 79.14.

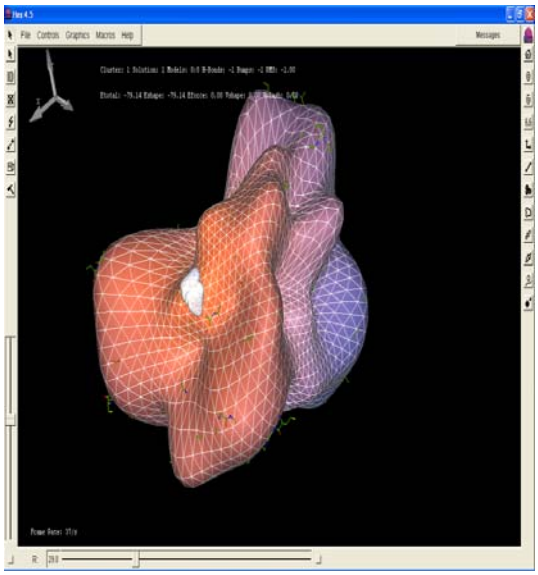

Figure 32 GEMCITABINE WAS DOCKED WITH MSLN USING HEX

MECHLORATHAMINE

Thus docking was done for our ligand mechlorothamine to our target mesothelin and its Evalue was found to be -51.05

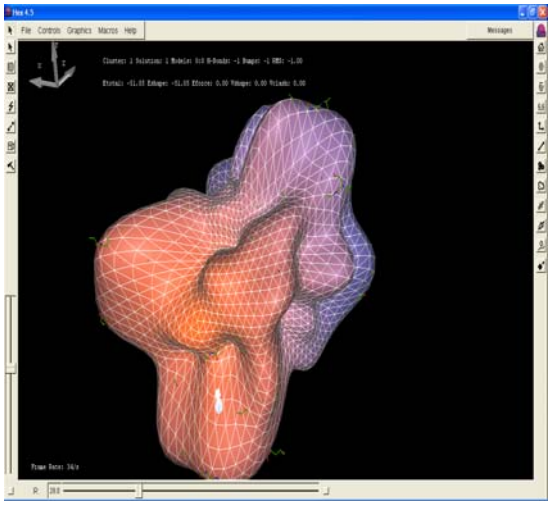

Figure 33 MECHLOROTHAMINE WAS DOCKED WITH MSLN

Letrazole

The Evalue of letrazole docked to our target mesothelin was foud to be -98.71.It was the highest docking score when compared to the other leads.

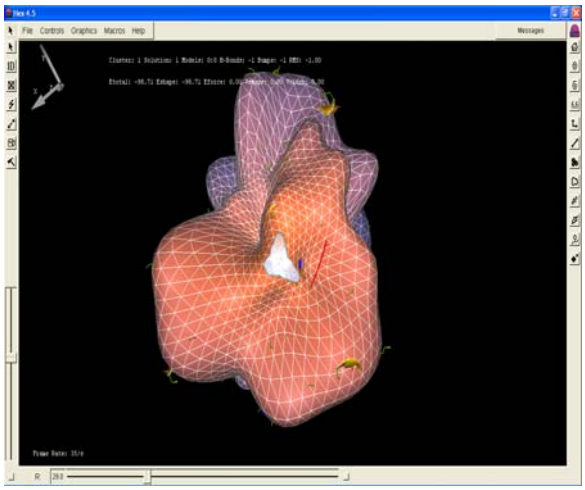

Figure 34 LETRAZOLE WAS DOCKED WITH MSLN USING HEX

CARMUSTINE

The Energy value of carmustine was docked with our target mesothelin was found to be –81.41 and it was the second highest docking score when compared to the other leads.

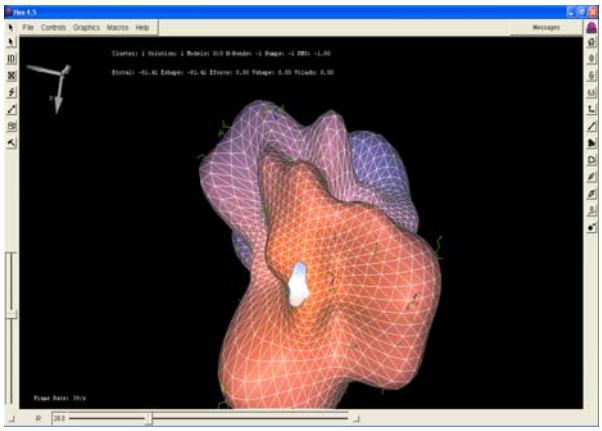

Figure 35 CARMUSTINE WAS DOCKED WITH MSLN USING HEX

On the basis of the highest docking score Letrazole and Carmustine was found to be the successful drug candidate for our target Mesothelin-Mesothelioma cancer. The energy value of these drugs using hex also confirm that drugs used for ovarian carcinaoma was desirable for mesothelioma cancer also.

7. CONCLUSION

The study of mesothelin gene in human mesothelioma cancer gave a clue that mesothelioma has been majorly evolved through the environment exposure of asbestos and hence of great importance in understanding the mechanism of cancer. The probable explanation could be the proteolysis of mesothelin gene leads to the release of soluble peptide in to the blood serum. The structure of mesothelin was solved theoretically using homology modeling. We believe that with some of the successful drugs for lung cancer such as Gemcitabine,Cyclophosphamide,Mechlorotham ine and for ovarian cancer such as Letrazole& Carmustine, would be forbide the over expression of mesothelin gene. The release of peptide from mesothelin was prevented by using the docking techniques such as autodock & hex. The binding energy of those drugs also

increases the confidence of using those ligands as candidate for this particular protein. Comparative analysis of these drugs was done, depending upon their binding energy of ligand with that of the active site. Hence our research proved that letrazole has splendid ability to dock our target protein mesothelin. Since mesothelin gene was also found to be involved in ovarian carcinoma, this gene would be of great interest which lends support in the consideration of Human Mesothelioma cancer, requiring further study and research.

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