

Antisense therapy for Haematological Malignancies

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The life and death of a cells in the body is determined by active death and survival proteins manufactured within the cell. These proteins are controlled by corresponding “genes” that are coded for within the DNA. This process is usually balanced so that when one cell dies another divides to replace the dead cell, keeping the cell numbers in equilibrium. Occasionally one of the survival genes is turned on at the wrong time, due usually to an alteration (mutation) in the DNA code (the elements of the code are called bases). New cells continue to be created by division, however, the older cells fail to die due to the presence of the survival protein. The result is an accumulation of cells, forming a tumor “lump” as seen in cancer and lymphoma patients (see fig. 1). If the gene can be identified then methods to “turn off” or “silence” this gene would help to kill the lymphoma cell (a process known as apoptosis). Recently, one such method has been successfully investigated and is called “antisense oligonucleotide therapy”.

The concept is relatively simple, however, in practice there have been problems in identifying the survival gene and in selecting the appropriate part of the mRNA to target. Over the last 4 years many of these technical problems have been overcome. The sequencing of the human DNA code opens up considerable possibilities for effective AS therapy in a large range of diseases.

The gene causing Follicular lymphoma, the BCL-2 gene (B-cell lymphoma gene number 2) was one of the earliest to be discovered. This gene prevents cells from dying, even when treated with high doses of chemotherapy. We now are aware that high levels of the BCL-2 protein are present in a number of additional cancers including other forms of lymphoma

and leukaemia. In general, where high levels of BCL-2 protein are present conventional treatment with chemotherapy is less effective. A BCL-2 AO therapy (Genasense – Aventis) has been devised and is hoped to have an anti-lymphoma effect. Such a therapy has been under experimental investigation in the laboratory and has now completed a number of trials in patients, with further underway. Over 2,000 patients have been treated to date with Genasense (GNS). This is a new and radically different approach to cancer therapy. Some of the background and problems involved are presented in this abstract.

What is “Antisense Therapy”?

The DNA code found in the nucleus (“inner core”) is read and turned into a protein with the help of a single strand of “sense messenger RNA”. Without the “messenger” the DNA code cannot be turned into a protein. Antisense oligonucleotides (AOs) are short (typically 13 to 20 bases in length) sequences of single stranded DNA that can bind very specifically to the sense messenger RNA (mRNA). When the AO binds to the mRNA an enzyme (Rnase H) within the cell is activated, “destroying” the mRNA. Having “shot the messenger” in the case of Genasense no signal is given to make the survival protein (BCL-2) and the malignant cell is made susceptible to death again.

The earliest attempts to inhibit gene expression by AOs were made as far back as 1978. However, the oligonucleotide had to be constructed manually, was very time consuming and impractical. In the 1980s technology was devised for automated synthesis of oligonucleotides, but, the backbone of the molecules failed to protect them from rapid destruction in the human

setting. This meant that huge quantities would be required for therapy. A simple change of the backbone chemistry from oxygen to sulphur (Thioate chemistry) was shown to dramatically lengthened the life of the molecule from minutes to days in the late 1980s and meant that the amount required was practical to treat patients. In addition, during the 1990s the manufacturing process was scaled up so that the costs of manufacture were reduced from tens of thousands of dollars to hundreds of dollars for each gram, enough to treat a patient for approximately two weeks. This opened up the prospect of gene silencing by synthetic AOs as a therapeutic tool for cancer.

A number of genes were targeted in a range of malignancies by selecting arbitrary sequence of the appropriate mRNA. This should have readily provided therapeutic molecules, but, in many cases the protein production from the gene continued unhindered. It became clear that not all of the mRNA sequence was capable of binding to AOs due to its complex structure. Methods had to be devised to create a “map” of each mRNA identifying the portions of the mRNA that accessible to combination with AOs. This was the major breakthrough and one of the first effective molecules to emerge was the BCL-2 AO (G3139, now known as “Genasense”, produced by Aventis).

How is antisense therapy given?

AOs dissolve readily in water and adequate amounts to treat the whole body for 24 hours can be contained in one or two teaspoons. This is conveniently administered through a vein over a period in the region of two hours. Alternatively it can be given as a continuous infusion under the skin, however, this may cause some skin irritation. There is some research work underway to try and make a form of AO that can be taken by mouth. In order to have the beneficial effect the treatment needs to be repeated on a daily basis and may be continued for up to three weeks. It can be restarted after a break of seven days. However if the main aim is to lower the protein (eg Bcl-2 protein) in the cell so that chemotherapy can be made more effective, daily infusions for 5 days is perfectly adequate. The AOs survive for a reasonable time so that a reasonable

level can still be detected at 48hours even after a single dose. In summary AOs are easily administered, relatively stable and long acting.

How does antisense get into lymphoma cells?

The AO molecule being small in size is able to circulate to most cells within the body apart from the brain. At the cell surface there is a protein that is capable of binding to AOs and then transports it from the outside surface to the inside of the cell. The amount getting into the cell is dependent on the dose given and the period over which it is administered.

What makes it specific to the lymphoma cells?

The AO does get into normal cells as well as lymphoma cells. However, it is only the lymphoma cells which are greatly dependent on the Bcl-2 protein for survival. Turning this protein off has drastic effects on the lymphoma cells ability to live, but in normal cells they are less dependent on the protein and can “switch on” alternative genes for survival (ie. They are “better organised”). Secondly the lymphoma cells have a greater capacity for small molecules to enter the cell, so accumulate higher levels of antisense compared to normal cells. Thirdly in many normal cells Bcl-2 is not present and understandably the AO cannot “switch off” what is not “turned on”. In this way Bcl-2 AO exerts a high degree of specific action against the lymphoma cell.

Does Bcl-2 antisense benefit lymphoma patients?

Genasense has now been administered to 2,000 patients with a range of malignancies. It was first thoroughly evaluated in a Phase I clinical study for lymphoma patients who had failed all other therapy (21 patients). All the patients had documented high levels of Bcl-2 protein in their lymphoma cells. The treatment consisted of the AO alone infused subcutaneously for a single course of two weeks. The dose in the first patient was very low and was increased with each subsequent

patient until a dose was reached that caused side effects. For such a new approach to therapy it was encouraging that the treatment was well tolerated with virtually no side effects until a dose well above that required to turn of Bcl-2 protein production (ie well above that required for therapy) was reached. The side effect at high dose was a fall in the platelet count and this is related to the sulphur chemistry of the oligonucleotide not due to the antisense sequence. This is reversed over a few days when the AO is stopped and no long term problems have been observed. As a therapy Bcl-2 AO was also promising with benefits to nearly half of the patients consisting of stabilisation of disease and one patient whose disease resolved and remains in this state some four years after completing a single two week course. However, of as much importance it was also possible to show that the Bcl-2 protein levels could be reduced in lymphoma cells. The most responsive patients were those who were able to demonstrate reduced levels of Bcl-2 protein. In summary, it has been shown that Bcl-2 AOs can be given safely to patients with lymphoma at levels that reduce the Bcl-2 protein and in some cases this leads to lymphoma stabilisation or regression with only a single two week treatment.

What happens next?

The prime aim of the Bcl-2 AO therapy for lymphomas has always been to make the tumor cells more sensitive to lower doses of therapy. The first step was to show that the Bcl-2 protein could be reduced in patient's lymphomas. The second step is to add in therapy. In fact this has now been tested out successfully in patients with malignant melanomas (these have very high levels of Bcl-2 protein and severe chemotherapy resistance). Trials for chronic lymphatic leukaemia, follicular lymphoma, mantle cell lymphoma, myeloma and acute myeloid leukaemia are currently under way. These studies administer the AOs for five to seven days to reduce the Bcl-2 protein and then administer the therapy. In the case of mantle cell lymphoma the therapy includes antibody therapy (Rituximab). Recent work combining

Genasense with other biologics including the proteasome inhibitor Velcade have suggested novel combinations for future clinical trials.

Bcl-2 AO therapy is a novel and logical approach to the treatment of haematological malignancies based on the tumor cell biology. While we have some way to go in optimally applying such biological therapies it offers a real hope that we may be able to improve survival in patients for whom chemotherapy has ceased to be effective. In addition it emphasises that new and more effective therapies must come from a greater understanding of lymphoma cell biology and the reasons why current therapy can fail. New technologies give us considerable hope for the not too distant future.

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