AGX51, a potential anti-tumour therapy targeting ID proteins

Cancer is challenging to treat. Dr Robert Benezra, Member of the Cancer Biology and Genetics Program at Memorial Sloan Kettering Cancer Center, has recently identified a biological mechanism that allows certain fatal cancers of the liver, brain and breast to regenerate despite their apparent elimination by surgery, chemotherapy or radiation. This mechanism, which enables their persistent recurrence and lethality, involves ID proteins. Together with AngioGenex, Inc., Dr Benezra has now identified a small molecule, AGX51, that inhibits ID proteins and disrupts the cellular pathways by which cancers repropagate, and could therefore be an important new anti-tumour therapy.

Dr Benezra’s recent findings are the culmination of 30 years of basic and translational research. Back in 1989, ID (“Inhibitor of DNA binding”) proteins were identified as inhibitors of cell differentiation. Since then, ID proteins have been shown to play a critical role during normal embryonic and fetal development. As the foetus develops and cells differentiate to acquire different characteristics and carry out specific functions, it is important that a pool of stem cells (undifferentiated cells) keeps its stem-cell state and maintains its self-renewal capacity. This is, in part, taken care of by ID proteins, which inhibit cell differentiation.

In healthy adult tissues, ID protein expression is largely silenced, meaning that cells are mostly devoid of ID proteins. However, ID protein expression is reactivated in a variety of human cancers. Over-expression of ID proteins in cancer is often associated with more aggressive disease and an increased risk of metastasis. Recent evidence suggests these proteins are present in resting cancer stem cells which resist standard chemotherapies and may be responsible for disease recurrence.

**HOW ID BLOCKS DIFFERENTIATION**

The primary mechanism of action of ID proteins is to isolate, or ‘sequester’ other proteins by interacting with them. Dr Benezra and his team have focused on the protein-protein interactions between ID and E proteins. E proteins induce cell differentiation. As transcription factors, E proteins interact with DNA and promote the expression of specific genes that will give the cell specific characteristics and stall their growth. ID proteins bind to E proteins and, by doing so, prevent the formation of active transcription complexes and block E-protein-mediated gene expression. As a result, cell differentiation is inhibited.

**ID PROTEINS IN CANCER**

Oncogenic factors that promote proliferation and cancer activate different signalling pathways that lead, among other processes, to the over-expression of ID proteins. ID proteins are highly expressed in many solid tumours, including breast, pancreas, bladder, uterus, colon, stomach, nervous system, liver, ovary, prostate, kidney, oesophagus, lung and thyroid as well as in leukaemia. In all these cancer types, the presence of ID proteins is often associated with more aggressive disease and an increased metastatic potential, resulting in poor clinical outcomes.

Because ID proteins are over-expressed in cancer, Dr Benezra’s work applies the hypothesis that an anti-ID therapy could be an effective treatment for cancer.

**ID PROTEINS IN VISION LOSS**

In cancer, new blood vessels are formed to vascularise the tumour, and ID proteins were shown to be required in the newly formed vessels. Given these findings, Dr Benezra hypothesised that reducing ID protein levels would be protective not only in cancer, but also in ocular neovascularisation-related pathologies, where the pathologic formation of vessels is also involved. The contribution of ID proteins in this context had at the time not yet been explored.

Wet age-related macular degeneration (AMD) and retinopathy of prematurity (ROP) are two examples of neovascularisation-related pathologies affecting the eye. As a result, retinal function is impaired. AMD and ROP are the leading causes of vision loss in adults over 60 and premature babies, respectively.

The team led by Dr Benezra showed, in mouse models of AMD and ROP, that the genetic loss of ID proteins is associated with less ocular neovascularisation. This finding confirms that ID proteins are involved in these pathologies, and also suggests that ID proteins could be a therapeutic target: an anti-ID therapy could be an effective treatment also for AMD and ROP.

**AGX51: AN ID DEGRADER**

Together with AngioGenex, Inc., Dr Benezra has recently identified a small molecule, AGX51, which acts as an antagonist of ID proteins. AGX51 decreases cell growth and viability and is a strong candidate for anti-tumour therapy.

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binds to ID proteins. This binding prevents ID proteins from interacting with E proteins, and effectively allows E proteins to carry out their function as transcription factors. In the presence of AGX51, E proteins modulate gene expression, promote cell differentiation and inhibit cell growth.

In the meantime, the binding with AGX51 destabilises ID proteins, which in turn leads to ID degradation. The interaction between ID and E proteins was formerly considered to be impossible to inhibit. AGX51 is therefore a first-in-class compound that may be useful in the management of multiple diseases.

**ACTION OF AGX51**

Notably, Dr Benezra and his team have managed to describe the molecular mechanisms involved in the binding of AGX51 to ID proteins, and the subsequent sequence of events that leads to the degradation of ID proteins.

In the absence of AGX51, ID and E proteins interact and form a complex called a dimer. The E protein is sequestered, unable to carry out its functions. AGX51 is a small molecule that can bind to a highly conserved region of ID proteins (a part of the structure that is very similar across all ID proteins). It’s important to note that in proteins, highly conserved regions are often important for folding, binding to other molecules, or for maintaining its structure.

The research team show that the interaction with AGX51 alters the ID protein structure, causing it to partially unwind. This structural change disrupts ID’s interaction with the E protein – allowing the E protein to be set free. The destabilised ID protein is then targeted for destruction by the cell. Importantly,
AGX51 reduced the tumour burden (the total amount of tumour, defined as the number of cancer cells or the size of the tumour).

More experiments, this time directly conducted on cancer cell lines, allowed the research team to study the effects of AGX51 from another angle. They reported that as ID proteins are degraded, molecules containing reactive oxygen species (ROS) are formed. As ROS build up in cells, they may cause damage to DNA and proteins, and eventually cause cell death. This explains how AGX51 decreases cell growth and viability and thus is a strong candidate for anti-tumour therapy.

A UNIQUE THERAPEUTIC APPROACH

Acquired resistance is one of the main challenges when it comes to treating cancer: even if a treatment is effective at first, cancer cells can eventually find ways to resist, making treatment ineffective.

Importantly, AGX51 holds promise as a therapeutic strategy because acquired resistance to this treatment has not been observed to date.

Cancer cells fail to acquire resistance to AGX51 because the molecule effectively short-circuits the two major pathways of acquired resistance. First, AGX51 binds to a highly conserved region of ID proteins. While mutations could be a way for cancer cells to develop resistance to AGX51 (so that AGX51 cannot bind to ID proteins anymore), they are very unlikely to occur in this region of the protein and still retain ID activity. Second, AGX51 quickly degrades ID proteins. Cancer cells can respond to the inhibition of a cancer-driving protein by producing more of that protein, but degraders like AGX51 can prevent such accumulation. With its strong anti-tumour effects and potential to bypass acquired resistance, AGX51 will be a molecule to watch.

View the mechanism of action of AGX51 in this animation: [https://www.youtube.com/watch?v=HmcwK89bZg](https://www.youtube.com/watch?v=HmcwK89bZg)

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AGX51 has no effect on the E protein. Freed from ID proteins, E proteins bind to DNA and exert their effects.

AGX51 TO TREAT AMD

Because an anti-ID therapy offers an exciting potential way to treat diseases such as AMD that cause vision loss, Dr Benezra and his team examined the effect of AGX51 in mouse models of AMD.

Their results show that AGX51 is well tolerated in mice, with no apparent toxicity. In fact, AGX51 has the same effect as the genetic loss of ID expression - levels of ID proteins decrease because the proteins are degraded, and pathologic retinal neovascularisation is inhibited. Excitingly, this confirms that AGX51 could be beneficial in the context of AMD.

AGX51: A POTENTIAL ANTI-TUMOUR THERAPY

After showing that AGX51 targets ID proteins for degradation and impairs ocular neovascularisation in mouse models, Dr Benezra and his team studied AGX51’s potential as an anti-tumour therapy.

In mouse models, they showed that AGX51 treatment stops breast cancer from spreading to the lung. The growth of chemotherapy-resistant breast tumours also regresses in response to AGX51 combined with chemotherapy. They observed similar results in a model of sporadic colorectal neoplasia, as development and their reactivation in a variety of cancers. Recent targeting of ID proteins by small molecules with the help of AngioGenex, Inc., has led to the development of novel targeted therapies being readied for clinical application.

References


Personal Response

AGX51 holds exciting potential for patients. Can you tell us more about future plans and potential clinical trials for this anti-ID molecule?

**We are currently amassing the safety and pharmacologic data required for filing an IND (Investigational new drug) with the FDA as a prelude to a first-in-human clinical trial. Cancers that express ID in resting stem cells have high relapse rates and poor prognoses. These will be the first cancers we will be treating with our new oral formulations.**