PROFESSOR EVANGELIA PATSAVOUDI

Anti-cancer antibody

Professor Evangelia Patsavoudi shares her research on a new potential cancer treatment, utilising an antibody that her own group manufactured called mAb 4C5. Following this groundbreaking work, her team is aiming to begin clinical trials

Could you discuss the background behind your latest project on proteins?

Our original goal was to identify new proteins involved in the development of the nervous system. We immunised mice with embryonic brain membranes and produced monoclonal antibodies, picking one out that we called mAb 4C5. We studied this antibody and eventually identified the protein this antibody recognised as being heat shock protein 90 (HSP90), which is involved in numerous activities of normal cells, but also in many cancer processes. We then decided to leave our research concerning the nervous system and focus our studies on inhibiting this protein in cancer processes.

What important outcomes has your research into anti-cancer antibodies produced?

We have demonstrated that our antibody affects metastasis of human breast cancer cells and mouse melanoma cells. Ongoing *in vivo* experiments indicate that mAb 4C5 also significantly delays primary tumour growth of triple negative breast cancer cells. Moreover, a unique and important characteristic of mAb 4C5 is that it is not internalised by the cell, thus it exclusively targets the cell surface pool of HSP90.

Can you elaborate on the relationship between your research group and the biotechnology firm Zestagen?

Zestagen was formed as a biotechnology start-up company around the discovery of our antibody. The goal of the company is to engage in full preclinical development (eg. humanisation, manufacturing process development, toxicology, etc.), such that mAb 4C5 enters phase I clinical trials.

Have you experienced any barriers during your work?

A frequent barrier in research is that after acquiring co-funding from EU and National Resources, there are many long and tedious bureaucratic procedures that we have to follow to be able to absorb the funding. Thus, experiments that need to be done quickly for us to remain competitive and obtain results are greatly delayed. Another important difficulty is finding capital for start-up biotechnology ventures. Disappointingly, potential investors are less and less prepared to invest in relatively early stage biotechnology projects, because of their inherent risks – ones that most new scientific research has – despite the fact that our work has shown solid results.

How do you envisage your research progressing?

Two important milestones in our drug development will be to humanise the chimeric antibody and improve its pharmacokinetic properties, such that the resulting preclinical lead can enter full manufacturing process development to produce a protein batch for toxicology experiments. Needless to say, the ultimate goal is for the antibody to enter phase I clinical trials. From an R&D perspective, we are also currently investigating whether HSP90 is expressed to a greater extent on cancer stem cells – the hard core cancer cells from which cancer and metastasis begins. Our goal there is to examine whether mAb 4C5 has an even greater effect on these cells in comparison to the non-stem cancer cells.

Taking control of metastasis

Preventing metastasis, the spread of a cancer from one organ to another, is a huge challenge in the field of oncology. Researchers at the **Hellenic Pasteur Institute** in Athens, Greece, are hoping their novel antibody will lead to a cure for metastasis in breast cancer and melanoma

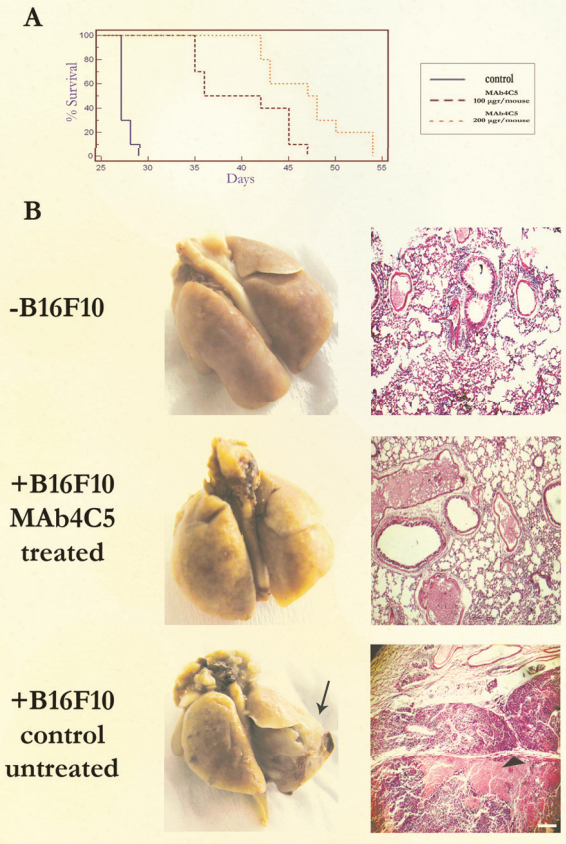
TUMOUR CELL METASTASIS – a complex, multistep process in which malignant cells detach from their point of origin, migrate and invade surrounding tissues – presents a major problem in the treatment of cancer because the cure rate is very low. There is a huge need for new therapeutic approaches. One validated pharmaceutical target is heat shock protein 90 (HSP90), part of a family of chaperone molecules. The protein is one of the most abundant in unstressed cells under normal conditions.

Evangelia Patsavoudi – Professor of Biology at the Technological Educational Institute (TEI) of Athens and Group Leader at the Department of Biochemistry of the Hellenic Pasteur Institute – discovered a monoclonal antibody, mAb 4C5, which specifically targets HSP90. On the back of this important finding, Patsavoudi is aiming to translate this research into a final product to cure people with cancer.

INNOVATIVE INVESTIGATIONS

Inside normal adult cells, HSP90 is present as a cytoplasmic protein that performs various housekeeping functions. These include protein folding, trafficking and activation – hence the term chaperone molecule. To date, academic papers on the protein have focused on the intracellular pool – that is, until Patsavoudi's curiosity pushed her to look a little closer at the cells' surface. What she found surprised her: "HSP90, though present on the surface of migrating cells during development, is hardly detectable on the surface of normal adult cells – they only express the protein inside the cell. In contrast, HSP90 is excessive on the surface of cancer cells, especially those that belong to highly aggressive cancers such as breast cancer and melanoma".

This revelation inspired Patsavoudi to investigate ways of using mAb 4C5 to target cancerous cells while leaving healthy, normal adult cells unaffected. If taken to market, this type of treatment would massively improve upon its predecessors, such as chemotherapy, which are non-specific treatments that indiscriminately kill off healthy cells along with cancerous ones.



Assay of melanoma metastasis.

A. Kaplan-Meier survival curves indicating significantly improved survival of mice treated with 100 µg (**dashed line**) and 200 µg (**dotted line**) of monoclonal antibody (mAb) 4C5 (P< 0.0001) when compared to control untreated mice (**solid line**). The survival rate of the mAb 4C5 treated mice was dose dependent (P=0.0075).

B. Lung autopsy of untreated dead and mAb 4C5 treated survived mice, 27 days after B16 F10 intravenous injection. In control mice, the presence of massive metastatic tumours observed at a macroscopic level (**arrow**) was confirmed at a microscopic level in hematoxylin-eosin stained sections of the corresponding lungs (**arrowhead**). In contrast, a mAb 4C5 treated mouse (100 µg/animal) sacrificed on the same day showed no melanoma metastasis and had the same profile as a mouse that had not been inoculated with B16 F10 melanoma cells. Scale bar =1,000 µm. Figure adapted from Stellas et al, 2007, *Clinical Cancer Research*, **13**, 1831-8.

A MIGHTY MONOCLONAL ANTIBODY

To demonstrate the worthiness of mAb 4C5, Patsavoudi needed to provide proof of its effectiveness through experimentation. Starting with *in vitro* models in a wound healing assay, she showed that mAb 4C5 significantly inhibits cell migration processes – and thus cancer cell invasion – by binding exclusively to cell surface HSP90 without being internalised. Following on, she used clonogenic assays to show that the antibody also inhibits the growth of clones, thus constraining cancer cell growth.

Patsavoudi and her group then moved to *in vivo* mice models. She observed that when mice predisposed with mouse melanoma cancer cells received mAb 4C5, their cancer cell metastasis was inhibited and their lifespan nearly doubled. These experiments showed that the results were dose dependent, as the control mice died on day 28, while mice treated with 100 µg of mAb 4C5 per day had nearly doubled their lifespan to 47 days, and mice treated with 200 µg per day lasted for an additional week. This verified the specificity of the antibody, meaning that it is unlikely the results were due to other reactions. Moreover, Patsavoudi then created a half-mouse, half-human form of the antibody and reproduced the experiments. "We have seen that this chimeric antibody has similar functional properties," she states. "This is a good sign that the antibody can be completely humanised in the future and retain all its anti-cancer activity."

BEATING BREAST CANCER

Patsavoudi is also demonstrating the anti-tumour growth effect of mAb 4C5 in human breast cancer cells. "We have ongoing *in vivo* experiments showing that this antibody not only has anti-metastatic properties but also significantly delays primary tumour growth of human triple negative breast cancer cells," she expands. This is an exciting outcome for her team, as it provides further evidence that the antibody can successfully be translated in human applications.

POSITIVE PROSPECTS

Patsavoudi's next aim is to develop a humanised version of mAb 4C5. "At the moment, we are looking for funding to humanise the chimeric antibody and thus result in a preclinical protein batch for toxicology experiments, eventually moving on into phase I clinical trials," she explains. Finding this funding has become the main objective of Swiss biotechnology company Zestagen, which owns the intellectual property rights of the antibody. Patsavoudi sits on the scientific advisory board of the organisation, which is dedicated to creating monoclonal antibodies against metastasis. As one of the few companies developing monoclonal antibodies against HSP90, Zestagen's aim is for its highly efficient operations to capitalise on the expanding market within the pharmaceutical industry. "Our overarching goal is to cure cancer," Patsavoudi enthuses. "With a humanised form of mAb 4C5, we believe we have a very good shot of achieving this."

INTELLIGENCE

TREATING CANCER WITH ANTIBODY MAB 4C5

OBJECTIVES

- To understand the mechanisms underlying the function of extracellular heat shock protein 90 (eHSP90) in cancer growth, invasion and metastatic processes using *in vitro* and *in vivo* models
- To investigate the possibility of using monoclonal antibody mAb 4C5 as a therapeutic agent for the treatment of both breast and melanoma cancer
- To study eHSP90 in cancer stem cells and investigate the possibility of using mAb 4C5 as a marker for these cells, and ultimately as a therapeutic agent

KEY COLLABORATORS

Dr Katerina Sidera; **Dr Dimitra Thomaidou**; **Dr Theodora Stivarou**; **Dr Avgi Mamalaki**, Hellenic Pasteur Institute, Greece • **Dr George Panayotou**, Biomedical Sciences Research Center, Alexander Flemming, Greece • **Dr Andreas Karameris**, 417 Veterans Administration Hospital, Greece

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EVANGELIA (LIA) PATSAVOUDI is Professor of Biology and Physiology at the TEI of Athens and a collaborating researcher at the Hellenic Pasteur Institute where she is a group leader. She has extensive biomedical research experience and has produced approximately 30 publications. After studying biology at the University of Patras, Greece, she completed her postgraduate studies and obtained her doctorate degree at the Pierre & Marie Curie University, France. Her current research interests lie in the field of cellular and molecular oncology and in particular on the study of extracellular HSP90 in cancer growth and metastasis.

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