Dr Liliana Soroceanu, a neuro-oncologist researching glioblastomas, explains her collaborative work with clinicians to develop novel therapeutics for improved patient prognosis.

Could you give an overview of your work at the California Pacific Medical Center Research Institute (CPMCRi)?

CPMCRi is the research arm of the CPMC, the largest medical centre in San Francisco. CPMC is a non-profit academic quaternary care hospital affiliated with Sutter Health, the largest healthcare system in northern California. My collaborator, Dr Charles Cobbs, and I started a neuro-oncology laboratory there that has unique access to brain tumour tissues from patients obtained under an institutional review board approved protocol.

What was the significance of Cobbs’ work linking cytomegalovirus proteins with brain tumours? Did you play a role in this early research?

Cobbs showed for the first time that a relatively ubiquitous human pathogen, human cytomegalovirus (HCMV), is found in over 90 per cent of human glioblastomas (high grade gliomas), a rapidly fatal type of brain tumour. I worked with him to investigate the mechanisms HCMV uses to modulate glioma biology and found that HCMV is expressed in glioma stem cells (the subpopulation of cancer cells believed to initiate tumours) and contributes to therapy resistance and tumour recurrence. The virus impacts specific signalling pathways important for cell survival and proliferation, as well as tumour cell invasion and angiogenesis.

How did you identify these mechanisms experimentally?

Among other methods, we used loss of function approaches by knocking down individual viral genes using siRNA technology and measuring the effects on tumour growth, invasion or angiogenesis depending on which process we hypothesised the viral gene was modulating. Conversely, overexpression of individual viral genes can uncover their role in oncogenesis. Another approach, used by our collaborator Dr Nino Chiocca at Harvard University, was to administer HCMV to a newborn mouse bearing a genetically induced glioma, which enhanced tumour aggressiveness and frequency (http://cancerres.aacrjournals.org/content/73/11/3441)

You have spent the past two decades working in brain tumour research. How has the field developed in this time?

The field has evolved and grown significantly in the past two decades. As in other cancer fields, much of the progress is based on the use of patient-derived tissues and primary cells grown in conditions that faithfully recapitulate the human disease. Working closely with clinicians, in my case neurosurgeons, is invaluable. Another major advance that has greatly benefited the brain tumour research community is The Cancer Genome Atlas (TCGA) initiative, which has undertaken molecular characterisation of human cancers. Glioblastoma was the first cancer to be ‘decoded’ by the TCGA consortium and this has led to better understanding of the molecular basis of the disease and the response to therapy, plus the design of novel, individual-led therapies.

Understanding the complex tumour microenvironment, including the possible role of pathogens in glioblastoma progression, and harnessing the immune system to fight cancer cells are both critical developments, which continue to impact brain tumour research and the search for better therapeutics.

Collaboration with similarly forward-thinking researchers has been a crucial aspect of your work. What has this brought to your endeavours?

I seek collaborators I can learn from. As such, I have worked with experts in cancer stem cells, transgenic models of disease, immunotherapy and virology, to name just a few. I learn from more experienced collaborators in grant writing strategies and the importance of moving science from bench-to-bedside in a timely manner, especially when we are targeting a disease with a current median survival of 14 months.

You are also exploring the world of personalised healthcare, looking at treating glioblastomas based on individual patients’ phenotypes. What progress have you made in this cutting-edge area of medicine?

So far we have profiled over 100 brain tumour tissues obtained from CPMC hospital patients with the help of our neurosurgeon colleagues. We used the published results from TCGA studies to classify these samples into different molecular entities, based on specific genomic and pathway alterations. The hope is to have targeted therapies tailored specifically for each individual patient tumour and be able to offer such treatment as soon as possible after diagnosis or surgery.

Looking to the future, we also hope to use biomarkers of glioma that ideally could be identified non-invasively from peripheral blood serum and used for screening, prevention and early intervention in brain tumour patients.
A virus to fight cancer

A combination of novel therapies developed by scientists at the California Pacific Medical Center in the US could transform diagnosis and lead to more effective, personalised treatments of aggressive glioblastoma brain tumours.

**Glioblastoma is the** most common form of primary brain tumour in adults and, unfortunately, the most aggressive. Despite combined treatments of surgery, radiation and chemotherapy, patient survival post diagnosis is severely jeopardised and death is almost inevitable. A common herpesvirus, human cytomegalovirus (HCMV, or herpesvirus 5) is associated with over 90 per cent of glioblastomas and there is mounting evidence to suggest that HCMV is involved in promoting tumour development.

Dr Liliana Soroceanu and her colleague, Dr Charles Cobbs, established a neuro-oncology laboratory at the California Pacific Medical Center Research Institute (CPMCRI) with the aim of investigating the link between HCMV and glioblastoma tumour formation.

The theory that a ubiquitous virus could be linked to a devastating cancer was initially met with scepticism from both oncologists and virologists; however, Cobbs' original paper linking the two was later confirmed by Drs Duane Mitchell and John Sampson of Duke University. As Soroceanu explains: "While HCMV may be a mere passenger of malignant glioblastoma, the well-established biological properties of this pathogen would suggest it could play a profound role in oncogenesis and tumour promotion."

**Mechanisms of Disease**

Soroceanu's research has shown that HCMV proteins can drive oncogenesis in three ways.

Firstly, HCMV activates platelet-derived growth factor-α (PDGFR-α) in neural precursor stem cells, preventing differentiation and enabling increased proliferation and accumulation of genetic alterations, potentially leading to tumours.

Secondly, as tumours grow they require their own blood supply. Soroceanu has shown that HCMV viral protein US28 induces the expression of vascular endothelial growth factor (VEGF), which promotes the formation of new blood vessels. Her team has also detected that HCMV protein pp71 in the majority of glioblastomas was responsible for an increase in stem cell factor (SCF) excretion, another important player for blood vessel formation in tumours.

Finally, HCMV promotes tumour invasion. Soroceanu and colleagues found that glycoprotein B, a viral protein, directly engages with PDGR-α to promote another of its functions: glioblastoma invasion.

**Virus Targets for Treatment**

The discovery of a viral connection to glioblastomas has prompted new treatments for this usually fatal disease and may even allow its prevention. "If HCMV is indeed involved in gliomagenesis, a viral aetiology of cancer could lead to preventive therapies similar to the use of HPV vaccine to prevent cervical and other cancers," Soroceanu elucidates.

Intriguingly, her collaborators at Duke University found that HCMV proteins can be found on the surface of glioblastoma cells but not on healthy brain tissue. They used these proteins as antigens to develop immunotherapy as a less toxic alternative treatment for the cancer and were able to prime T cells, part of the immune system, to respond specifically to HCMV protein pp65 and thus target glioblastoma cells specifically.

The link between glioblastomas and HCMV allows researchers to exploit antiviral drugs that have already been approved for clinical use, hopefully speeding up the process of treatment. Cobbs and Soroceanu's team worked with Dr David James at the University of California, San Francisco (UCSF), to trial cidofovir, an antiviral drug already approved for the treatment of HCMV retinitis in patients with AIDS.

When treated with cidofovir and ionising radiation treatment (RT), mice bearing human glioblastomas implanted within their skulls survived significantly longer than those receiving RT alone. HCMV gene expression was inhibited and glioblastoma cells underwent apoptosis, a type of cell death. Interestingly, these results were obtained independently of HCMV infection. The cidofovir promoted double-stranded DNA breaks in the tumour cells, and may have acted to sensitise cells to radiation therapy.

In another clinical trial, researchers at the Karolinska Institute gave patients another antiviral, valganciclovir, in addition to standard glioblastoma therapy. The median overall survival was 25 months, almost twice as long as the control group. This remarkable result...
is an encouraging sign that antivirals would make a beneficial addition to the current array of glioblastoma treatments.

CANNABINOIDS REDUCE GliOBlastoma INVAsIVENESS

Despite advances in glioblastoma treatments, these tumours are almost universally fatal within five years. The aggressive migration and metastasis of glioma cells means the cancer rapidly spreads to healthy parts of the brain. In 2012, Soroceanu and her collaborators found that a known breast cancer metastasis gene, ID-1, plays a critical role in regulating the invasiveness of glioblastoma cells. As well as decreasing invasion levels, ID-1 expression knockdown reduced the stem cell markers expressed by the glioblastoma cells.

Previous studies by Dr Sean McAllister have shown that cannabidiol (CBD), a non-psychoactive cannabinoid, was an effective inhibitor of ID-1 expression in breast cancer cells. Together, Soroceanu and McAllister used cannabidiol to downregulate ID-1 expression in glioblastomas and reduce their associated invasiveness. Soroceanu expanded on the benefits of CBD: “Other studies have demonstrated the anti-cancer effects of cannabinoids, however those studies have focused on Δ9-tetrahydrocannabinol (THC), the plant ingredient which does have psychoactive properties and as such, only limited quantities can be used. CBD appears equally effective to THC and has the added benefit of not exerting undesirable side effects”.

Soroceanu is currently working with oncologists from UCSF and the UK to set up a clinical trial for glioblastoma patients using CBD. She is very hopeful that it will make a difference to patient therapies: “Since CBD is both non-toxic and can easily access the brain tissue, we believe it is a lead drug candidate for adjuvant therapy in glioblastoma patients”.

LOOKING TO THE FUTURE

Glioblastomas could be difficult to treat because the tumours are formed of many different cell types, and current aggressive treatments combining surgery, chemotherapy and RT still result in poor patient prognosis. For this reason, Soroceanu and colleagues are looking at ways to combine newly developed therapies to improve patient survival, based on the molecular phenotype of the individual’s tumour. Combining published data from The Cancer Genome Atlas (TCGA) with the profiles of over 400 brain tumour tissues, Soroceanu may one day be able to design tailored therapeutics for each patient. Mitchell and Sampson amplified HCMV gene products from the blood of glioblastoma patients; however, these were not present in healthy blood donors. In the future, she aims to use these biomarkers to develop a non-invasive blood test for glioblastomas, which could give patient-specific information for use in personalised therapeutics.

After two decades in brain tumour research, Soroceanu is as keen as ever to find an effective treatment for glioblastomas: “I hope that my research will continue to uncover basic mechanisms of brain tumour pathogenesis, which can be exploited to design novel therapies. Working in this field, one is always aware of the urgency of finding a treatment for a devastating cancer which affects many individuals”.

The discovery of a viral connection to glioblastomas has begun to lead to new treatments.