The new hierarchy of cancer research

Dr Galina Botchkina outlines the principles and direction of her latest project, which tests new anti-cancer drugs in line with recent advances in the understanding of tumour formation

To begin, could you outline the main research aims of your current project? What led you into this field of cancer research?

Since the mid-20th Century anti-cancer drug development has focused on rapidly proliferating cells. Recent evidence has convincingly confirmed that the majority of tumours are organised hierarchically and that a population of tumour-initiating or 'cancer stem cells' (CSCs) is responsible for tumour development and resistance to treatment. The main research objective of our team is to develop more effective and less toxic anti-cancer drugs, which target CSCs and not just bulk tumour cells. Currently, our research is focused on the evaluation of the therapeutic efficacy of two proprietary drugs; a new-generation taxoid (SBT-1214) and a novel synthetic curcuminoid (CMC2.24). We test these drugs against CSCs isolated from prostate and colon cancers, using relevant experimental conditions to allow for unbiased evaluation of drug effectiveness.

When were CSCs discovered and how do they differ from other stem cells and bulk tumour cells?

The idea that cancer is driven by cells with embryonic features (stem cells) has appeared periodically during the last 150 years. In recent times the failure of standard anti-cancer therapies to treat tumours has led to the revival of the stem cell theory of carcinogenesis. However, the absence of specific molecular markers and sensitive instruments did not allow for the detection and isolation of CSCs. Evidence was first documented in leukaemia in 1994 and now CSCs have been discovered in all major types of human cancers. The most significant difference between normal and cancer stem cells is a loss of the tight control and regulation of CSC proliferation.

By what means are tumours organised in a hierarchical fashion? Could you explain the role of CSCs in the formation of tumours and in cancer metastasis?

Tumour hierarchy is a fundamental concept in tumour biology, which promises novel cellular and molecular targets for modern anti-cancer drug development. Malignant tissues contain a minor population of stem cells at the top of the hierarchy. Taking into account that stem cells in general are an immortal/long-lived population, it is more realistic to suggest that only stem cells have enough time to accumulate the necessary mutations for their malignant transformation and initiation of a tumour. Importantly, CSCs are also recognised as a cause of metastasis, which is the reason for ~90 per cent of cancer-related deaths. Although metastasis depends on multiple factors, it is conceivable that CSCs are the only cell population with unlimited tumour-initiating potential and inherent plasticity to survive in a foreign environment.

How well established is it that CSCs are resistant to chemotherapy? What are the mechanisms that induce resistance?

In general, stem cells are evolutionary predisposed to survival under unfavourable conditions. However, several different mechanisms can potentially contribute to CSC drug resistance, including over-activation of multiple developmental pathways; high levels of expression of the relative quiescence CSCs, allowing them to escape the toxicity of drugs that target highly proliferative cells; the up-regulation of anti-apoptotic family members; the down-regulation of pro-apoptotic machinery; and their profound capacity for DNA repair.

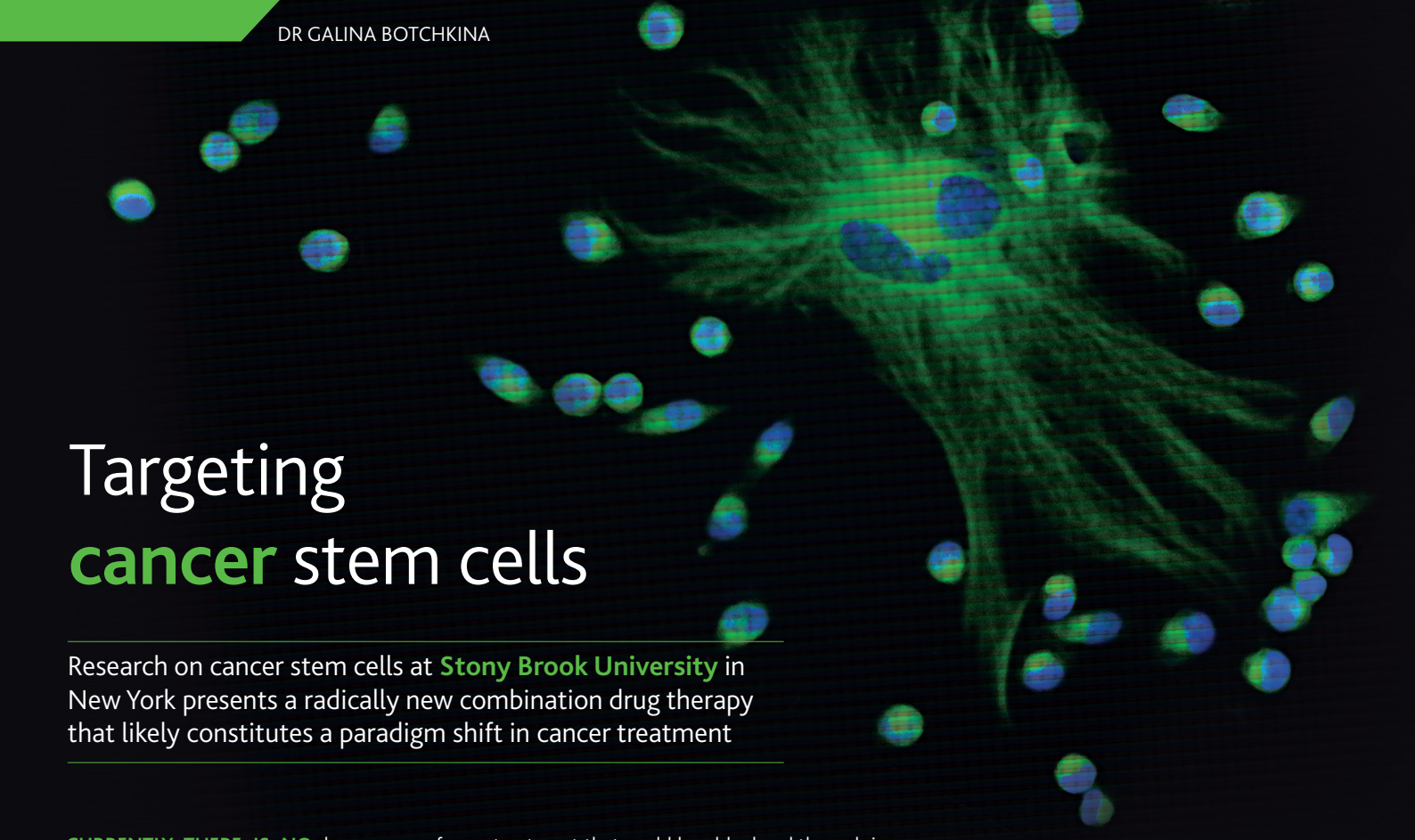
In what capacity will CSCs be targeted in developing an effective treatment for cancer?

Cancer development and progression to metastatic stages, as well as resistance to treatment and disease relapse, are associated with the deregulation and sustained activation of multiple tumourigenic pathways and genes that are regulated by multiple transcription factors (TFs). Our recent studies revealed that an SBT-1214/CMC2.24 combination effectively suppresses multiple stem-cell-relevant genes and TFs, which results in the successful suppression of *in vivo* tumours. In our analyses of the drug-induced alterations, we focus on CSC-enriched cell populations, which are proven to be highly tumourigenic, clonogenic and highly drug-resistant.

In what direction do you envision your research progressing? Are there any long term goals that you hope to achieve?

We have to continue isolation and biomolecular characterisation of the tumour-initiating cells from patient-derived tumour specimens. Purified and functionally characterised CSCs should be propagated in amounts suitable for thorough molecular analyses, and to serve as *in vivo* and *in vitro* models for preclinical evaluation of anti-cancer drugs.

Key collaborators Drs Iwao Ojima, Lorne Golub and Francis Johnson

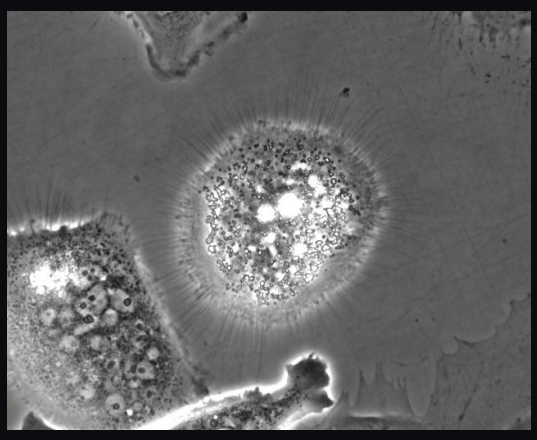


Targeting cancer stem cells

Research on cancer stem cells at **Stony Brook University** in New York presents a radically new combination drug therapy that likely constitutes a paradigm shift in cancer treatment

CURRENTLY THERE IS NO known cure for advanced epithelial cancers that represent the majority of human malignancies and are a major cause of cancer-related mortality. Two of the most deadly forms of these are colorectal and prostate cancer, which represent the second and third leading causes of cancer-related death respectively. Indeed, worldwide they are directly responsible for hundreds of thousands of deaths every year.

For more than half a century, anti-cancer drug treatment has focused on rapidly dividing cancer cells that make up the bulk of malignant tumours. Despite some evidence of increases in five-year survival rates, overall cancer mortality outcomes remain largely unchanged. In recent years however, advances in the understanding of tumour formation, hierarchy, and stem cell biology have offered new approaches to cancer treatment that could herald a breakthrough in cancer treatment and perhaps, even a cure to these devastating diseases.



CD133-positive prostate cancer cells.

CANCER STEM CELLS (CSCS)

Experimental and clinical evidence has confirmed longstanding theories that most tumours contain CSCs that are responsible for cancer initiation, development, maintenance and exceptional drug resistance. Normal stem cells have the capacity to create any cell type and consequently create any of the body's tissues. Similarly CSCs can form all of the heterogeneous tumour cell types but, moreover, they have an unlimited and unregulated capacity for self-renewal. It is now widely accepted that CSCs are the major driving force behind not only tumour creation but also the process of metastasis, which spreads cancer around the body.

In 2005, CSCs were shown by scientists to be present in prostate cancer (PrC) and two years later in colorectal cancer (CRC). Following the direct demonstration of their presence in intact, undisturbed tumours in 2012, the debate over their existence ended and the focus of research has now shifted to the targeting of CSCs for cancer treatment. Critically, it has been shown that many conventional anti-cancer drugs are not only ineffective against CSCs but can actually stimulate the proliferation of CSCs and drug-resistant cells. It is clear therefore, that CSCs represent one of the most crucial targets in the treatment of cancer.

Gigantic multinuclear prostate cancer cell expressing nestin – a neural stem cell marker.

ON THE RIGHT LINES

A pioneering project at Stony Brook University (SBU) in New York is underway with the aim of developing new, effective and less toxic anti-cancer drug treatments specifically targeting CSCs. Dr Galina Botchkina's group has established and characterised patient-derived cancer cell lines with enhanced CSC content, allowing for clinically and physiologically relevant models on which to test the combinational use of a new generation taxoid (SBT-1214) and a novel synthetic curcuminoid (CMC 2.24). "Historically, most drugs are tested on unselected cancer cells, using established cancer cell lines, which have low relevance to the original tumours," outlines Botchkina. "The progress of stem-cell research depends on the ability to grow stem cells in culture, however, there are well-known difficulties in growing primary human cancer cells *in vitro*."

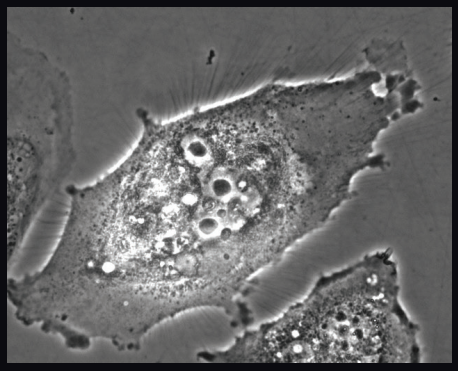
NEW TARGETS

The CSC models are providing an unbiased preclinical platform on which to evaluate anti-cancer drugs and are overcoming the limitations of cell lines that do not reflect the natural hierarchy of tumours, with a population of CSCs at the top-end. Moreover, there are vast differences between the gene and protein expression of regular cancer cells and CSCs that require a new approach. "Theoretically, there are many possible molecular targets to suppress or even eradicate CSCs," explains Botchkina.

Most tumours contain CSCs that are responsible for cancer initiation, development, maintenance and exceptional drug resistance

"A number of synthetic and natural inhibitors of multiple developmental and stem-cell-relevant signalling pathways are currently under intensive investigation."

Using highly drug resistant CD133- and CD44-positive prostate and colon cells isolated from clinical specimens, the team has already demonstrated the efficacy of SBT-1214 in combating colon and prostate cancers. Through both *in vivo*, xenograft mice models, and *in vitro* testing, the team had shown in a previous study that the new generation taxoid, SBT-1214, developed by Dr Iwao Ojima's Institute of Chemical Biology and Drug Development, is able to target and suppress CSCs.



Multinuclear CD133-positive prostate cancer cell.

THE SPICE OF LIFE

Building on this success, the group searched for a safe agent that could improve the CSC targeting activities of SBT-1214 and decrease the systemic toxicity associated with the use of the anti-cancer drug. Intrigued by the anti-cancer properties of the natural phytochemical curcumin – diferuloylmethane, the team began investigating its possible use in combination with SBT-1214. Curcumin is the principal curcuminoid found in the common indian spice – Turmeric. As Botchkina elucidates, turmeric has long been associated with health benefits: "Curcumin has been used extensively in Ayurvedic medicine for centuries. It is well-known, for example, that although prostate cancer affects many men in Western society, it rarely occurs in Japan, China or India, suggesting the existence of some epidemiological factors such as diet".

Indeed, curcumin has been shown to be antiproliferative, anti-invasive and anti-angiogenic, crucially, inducing apoptosis leading to the cancer cell death. However, due to the low bioactivity and bioavailability of curcumin, the research groups of Drs Francis Johnson and Lorne Golub at SBU have developed several synthetic derivatives, the lead compound being CMC2.24, which displays the same anti-cancer properties. Further *in vitro* studies using the combination of SBT - 1214 and CMC2.24 have presented very promising results.

A WINNING COMBINATION

The researchers have found the combination of SBT-1214 and CMC2.24 to be vastly more effective than either of the compounds individually. Using the highly tumorigenic and drug resistant cells expressing high levels of stemness markers CD133 and CD44, Botchkina's research group has demonstrated that the combination has profound pan-inhibitory effects on the transcription of CSCs and crucially, can induce the expression of pro-apoptotic and tumour suppression proteins previously absent in CSCs.

Known as the 'gene wakeup' mechanism, the presence of proteins, p53 and p21, is beginning to demonstrate the advantages of combinational drug therapy. "Activation of the down-regulated apoptotic machinery can increase sensitivity to other anti-cancer drugs, which can potentially reverse drug resistance," Botchkina expounds. "However, the mechanism induced by the drug combination should be further studied and tested *in vivo* against different types of cancer." Moreover, the two compounds in unison are likely capable of reducing side effects to chemotherapy treatments and amplifying the toxicity of diverse chemotherapeutic drugs to malignant tumours.

THE FUTURE OF CANCER TREATMENT

This research is truly breaking new ground. Indeed, the implications of these findings could resonate through the entire spectrum of cancer research, from the way we view diet as a means of prevention through to the treatment of end stage conditions. As Botchkina predicts, the new combinational therapy could hold the key to a cure for even the most deadly epithelial cancers: "We strongly believe that the demonstrated *in vitro* CSC-targeted activities of our SBT-1214/CMC2.24 combination will be expressed in a long-term suppression or even eradication of prostate and colon cancers *in vivo*". As more is understood about the role of CSCs, the work at SBU is offering the possibility of a paradigm shift in cancer treatment that could save the lives of millions of people worldwide.

INTELLIGENCE

COMBINATION OF A NEW-GENERATION TAXOID SBT-1214 WITH SYNTHETIC CURCUMINOID, CMC2.24

OBJECTIVES

To carry out a preclinical evaluation of a combination of a new-generation taxoid SBT-1214 with synthetic curcuminoid, CMC2.24, using clinically- and cancer stem cell-relevant prostate and colon cancer models. The study will determine the cellular and genomic alterations induced by this drug combination against functionally significant prostate and colon cancer stem cells (CSCs), not just bulk tumour cells. As a result of this project, a potential novel anti-cancer drug against currently incurable androgen-independent prostate cancer and colon cancer can be identified.

KEY COLLABORATORS

Stony Brook University:

Distinguished Professor Iwao Ojima, Director of the Institute of Chemical Biology and Drug Development, Department of Chemistry

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Distinguished Professor Lorne Golub, School of Dental Medicine

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GALINA BOTCHKINA obtained her MSc and PhD in human physiology and biophysics from the Lomonosov Moscow State University, Russia. She completed her postdoctoral training in neurobiology and molecular biology at the University of California, San Francisco. Botchkina is currently the Director of the Cancer Stem Cell Laboratory at Stony Brook University. Her current research focuses on tumour-initiating, or cancer stem cells (CSCs) and CSC-targeted drug development.

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