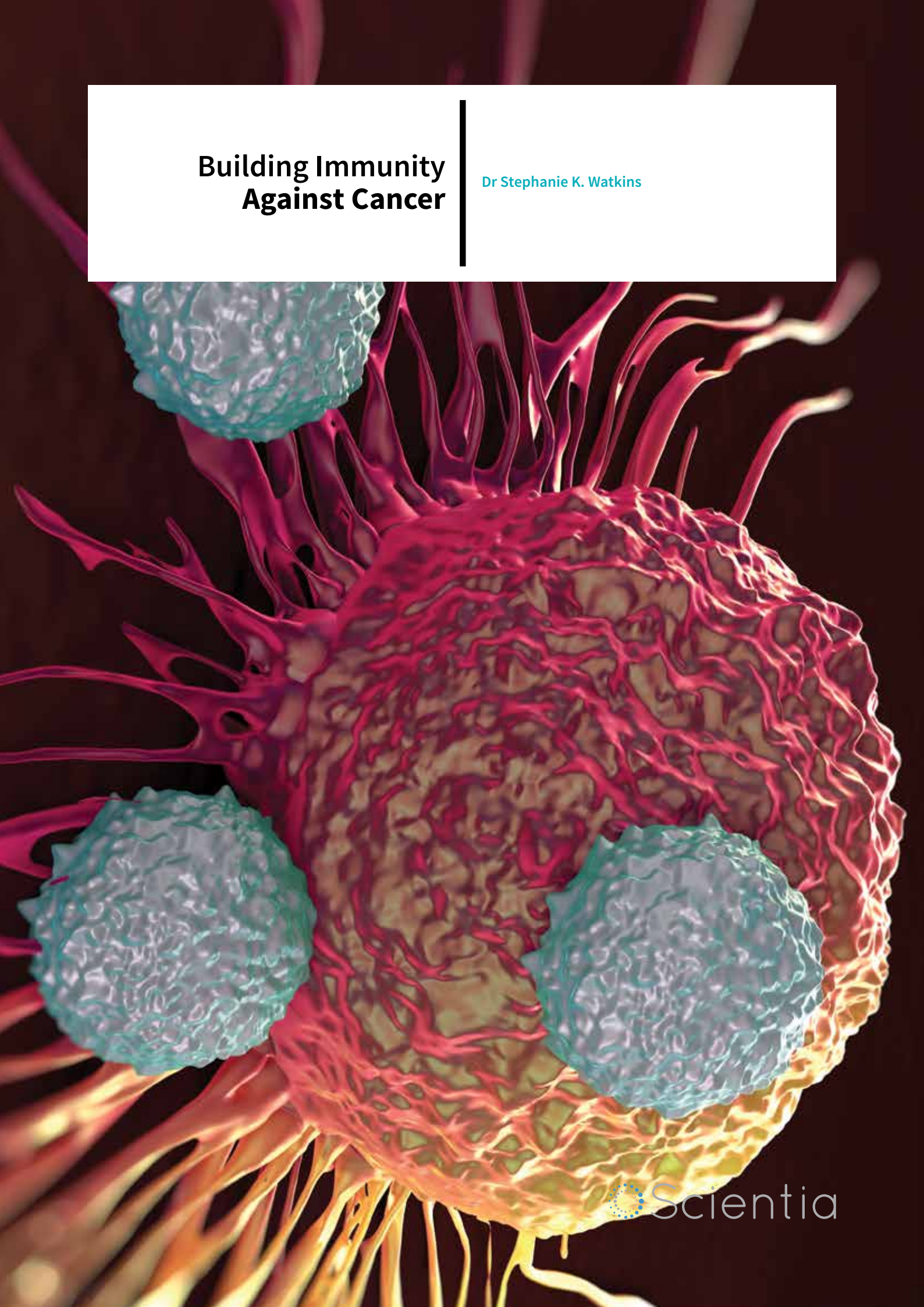
Building Immunity Against Cancer

Dr Stephanie K. Watkins



Scientia

BUILDING IMMUNITY AGAINST CANCER

A growing body of evidence supports how harnessing the power of the immune system may be the ultimate way to fight cancer. Therefore, researchers such as **Dr Stephanie Watkins** at Loyola University Chicago have been striving to increase our understanding of cancer immunology, by exploring the role of gender on immune cell activation. This fascinating research may lead to the development of new targeted cancer immunotherapies.

Cancer Complexities

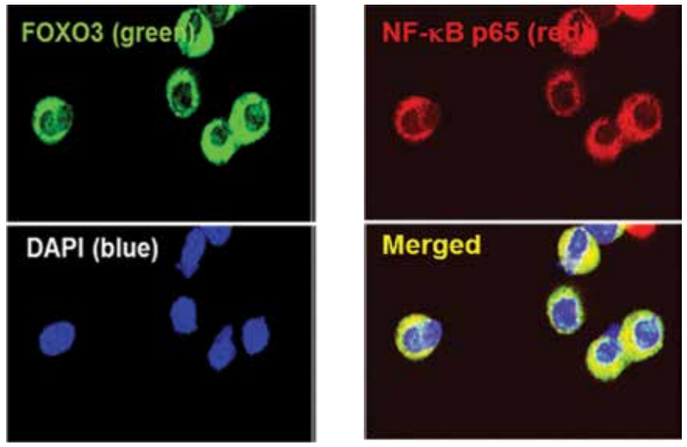
For every 100,000 people, there are around 455 new cases of cancer reported every year, while about 171 people die due to this terrifying disease. For this reason, oncology is an extremely important field of research, receiving much deserved attention from both researchers and governmental authorities. Cancer can arise in a number of different ways, each involving the hijacking of multiple pathways. Thus, cancer therapies must enrol and target a wide range of systems in order to be as effective as possible.

Every single day, a large number of cells in our bodies are damaged by internal and environmental factors. Some of these cells cannot repair themselves effectively and die out, while others survive despite their damaged DNA and produce new generations of cells with incorrect information. When enough of these so-called mutations appear in a single cell, the mechanism that ensures our survival transforms into one of the deadliest known diseases, where cells become virtually immortal and start multiplying out of control. Every day, it is up to our immune systems to clear up damaged cells with oncogenic potential to preserve our health.

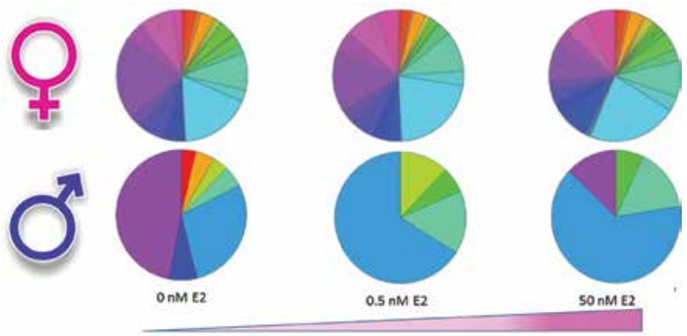
Yet tumour cells are not always recognised by the immune system, which enables them to escape destruction. Tumours have been found to create microenvironments that suppress immune response, where anti-tumour activity and the function of T cells are repressed. Dendritic cells play a critical role in our immunity against tumours because of their ability to trigger a strong immune response to the abnormal substances – or antigens – produced by cancer cells. Although these cells produce chemical signals to enable the recognition of tumours, they can be tricked into tolerating the tumour by the tumour microenvironments and therefore fail to send warning messages. Dr Stephanie Watkins at Loyola University Chicago investigated this phenomenon, specifically looking at a transcription factor known as FOXO3. FOXO3 is a protein class specific to humans, belonging to a family of transcription factors that play roles in regulating cell death – malfunctions of the FOXO3 system lead to dendritic cells becoming more tolerant of tumours. However, this relationship was found to be rendered more complex by the gender of the patient. Dr Watkins and her collaborators showed that there are significant differences between males and females in the frequencies and function of dendritic cell sets that infiltrate tumours. More precisely, the function of FOXO3 in dendritic cells was shown to be affected by exposure to oestrogens or androgens, which may lead to differences in cancer incidence, aggressiveness, and outcomes between genders.

In addition to inter-gender differences, cancer evolution is also known to be also influenced by alcohol, which reduces the ability of dendritic cells to react to antigens and promotes inflammation in response to trauma and infections. Despite its known role, the influence of alcohol on anti-tumour response had not previously been explored. Furthermore, vaccines for triggering a dendritic response are often produced from blood taken from the same individual, which means that previous exposure to alcohol can play a role in the efficacy of the vaccine. In a study examining the gender-dependent impact of alcohol on anti-tumour immune response, Dr Watkins and her team found differences between the signalling dendritic pathways of males and females, while also discovering that these differences are amplified by exposure to alcohol. In the team's experiments, female mice that were given alcohol showed reduced levels of granzyme B and interferon gamma (both having roles in immunity) and decreased function of CD44 and CD69 (two proteins found on the membranes of healthy cells). This showed that the female mice were unable to activate antigen-specific cytotoxic T cells and obtain immunity against the tumours. Furthermore, the alcohol countered even the extra FOXO3 produced by dendritic cells in response to the threat.

'I am interested in identifying the role of gender and sex hormones on immune cell activation and the outcome of targeting these pathways to enhance the activation of the immune response against cancer'



Tumour infiltrating dendritic cells (DC) express FOXO3 – mediates DC induced T cell tolerance/suppression.



Human tumour antigen specific cytotoxic T cell polyfunctional profiles in response to estrogen. Each color of the pie chart represents a population of T cells that produce a different combination of cytokines (TNFa, IL-2, IL-4, IL-17, IL-22, and IFNg or have lytic function as determined by CD107a).

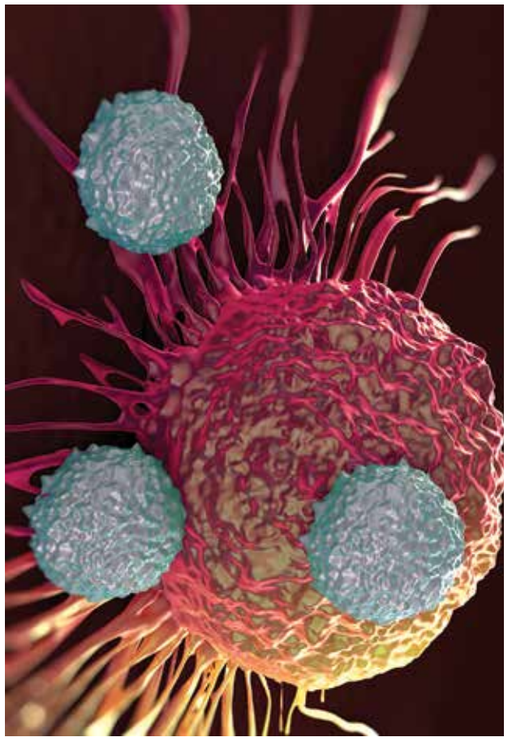
The Work of a Cancer Researcher

In her early career under the mentorship of the late Dr Robert D. Stout, Dr Watkins investigated the permanently changing behaviour of macrophages, a type of large white blood cells that migrate through the body engulfing and digesting foreign compounds, cellular remains, cancer cells, and microbes. This process is called phagocytosis, and healthy cells avoid this fate due to their surface proteins, which macrophages can recognise. Dr Stout was one of the first immunologists to recognise and thoroughly demonstrate that macrophages have the unique impressive ability to continually change their functions in response to their changing environment. Based on this, Dr Watkins found that macrophages which infiltrate lung tumours secrete anti-inflammatory cytokines and growth factors and are extremely immune suppressive. After injecting IL-12, or interleukin 12, into the tumour, Dr Watkins found that the macrophages quickly changed their behaviour – they produced a signalling substance to alert the immune system to the presence of cancer. She also discovered that the cells continued to change their function according to all the new stimuli they encountered – a process which persisted even after they were extracted from the cancerous microenvironment of the tumour.

Later on, Dr Watkins found that when macrophages infiltrating tumour sites release cytoplasmic interleukin 15, a process is initiated that leads to tumour regression. They knew that although anti-tumour vaccines can increase immunity in healthy mice, benefits in ill mice have been found to be minimal, due to the strong immunosuppressive environment created by the tumours in the host bodies. Although previous studies had tried to destroy the cells with immune-suppressing roles, success had been extremely limited. However, some scientists had reported that injecting interleukin 12 in mice bearing tumours initiates tumour destruction even if the mice have not been vaccinated to increase their immunity. Therefore, Dr Watkins injected interleukin 12 into mice to encourage leukocytes to invade cancerous tissues. Within two hours, they noticed that serum interleukin 15 increased, while the cytoplasmic interleukin 15 of the macrophages in the tumour decreased. The reverse was also true: injecting anti-interleukin 15 within an hour before interleukin 12 counters the beneficial effects – leukocytes are no longer interested in infiltrating the tumours and the reduction of the primary mass and clearance of metastases under the influence of interleukin 12 no longer occurs. However, injecting anti-interleukin 12 18 hours after interleukin 12 had no detectable impact of the activity of leukocytes. Together with the team's previous studies, this research proved the beneficial impact of interleukin 12 and its potential to

reduce the cancer-supporting behaviour of macrophages in tumours. The results suggest that resetting the function of macrophages is important for triggering the correct immune response to fight tumours, and deprives cancer of the growth support it normally receives. If integrated into current cytokine therapies, this finding has the ability to increase success rates and improve patient prognosis. For this work, she received her PhD from the University of Louisville in 2007.

With the knowledge that bone marrow, macrophages, and dendritic cells associated with tumours become hijacked and change their protective behaviours into immune-suppressive ones, Dr Watkins and her collaborators further looked into the pathways controlling myeloid cell function. In their experiments, they identified a direct interaction between two proteins, FOXO3 and NF-kB RelA. While FOXO3 regulates dendritic signalling, the NF-kB protein family (nuclear factor kappa-light-chain-enhancer of activated B cells) is present in most cells and controls DNA transcription, cell death, and the production of cytokines. Its role is to control the response of cells to external stress, for example UV radiation, free radicals, microbe antigens, or heavy metals. RelA, in particular, belongs to an NF-kB group responsible of transactivation, or an increase in the rate of gene expression. Dr Watkins found that FOXO3 binds NF-kB RelA in the cytosol – the liquid part of the cell cytoplasm. Consequently, neither protein can migrate to complete its activity, and instead, they remain locked near the location where proteins become attached to the transcription factor. However, when deleting the FOXO3 sequence, the NF-kB RelA resumes its activity. Although researchers are momentarily unable to suggest the consequences and applications of this finding, the study is important for being the first attempt in exploring the activity and roles of two proteins known to have a direct role in cancer propagation.



Future research directions

Dr Watkins' main research goal is to enhance immune based therapies for cancer by targeting pathways that regulate immune tolerance. At this point, she has three projects on the table that focus on advancing her previous cancer research.

The first project seeks to gain insight into the role of hormone receptor signalling in dendritic cells infiltrating tumours. Having identified the regulating role of FOXO3, which interacts with sex hormone receptors, she completed a preliminary study on mice and found that interventions targeting these transcription factors increased immunity towards tumours. The other finding was that decreasing the FOXO3 levels in female mice with tumours increased the growth rate of the tumours by approximately 4 times, a phenomenon accompanied by a lowered immune response. Although current therapies involve hormone receptors, the effect of targeting these pathways is not well understood at this point. Once Dr Watkins uncovers the impact of tuning the hormone receptor response, these findings will enable researchers to leverage the phenomenon and obtain better patient outcomes.

The second project is an inter-university collaboration between Loyola and Notre Dame, intended to produce molecule inhibitors that will break the interaction between FOXO3 and NF-kB – a transcription factor with a role in stress response and triggering immune reactions to infection. When incorrectly produced, NF-kB is linked to inflammation, cancer, and autoimmune disease. The research collaboration works with the premise that interrupting the FOXO3–NF-kB interaction promotes the immune potential of dendritic cells, and has applications in prostate cancer therapies.

'We found that dendritic cells that entered tumours had an upregulated expression of the transcription factor FOXO3'

Dr Watkins' third project focuses on understanding the multiple functions of T cells in melanoma and vitiligo, the latter being a condition predominantly affecting women, in which skin patches lose their normal colour. By means of polyfunctional cytokine analysis, her team found that certain patterns of cytokine production by T cells are linked with the appearance of vitiligo. Currently, the team is looking to understand whether the same patterns are responsible for ensuring immunity against melanoma. Dr Watkins' current graduate student and PhD candidate, Ms Flor Navarro, recently discovered significant differences in human male and female T cell polyfunctional profiles, especially upon stimulation in the presence of oestrogen. Further analysis will be required to determine the impact of therapeutically targeting these distinct patterns of function to control tumour growth and simultaneously prevent autoimmune disease.

Meet the researcher

Dr Stephanie K. Watkins
Assistant Professor
Department of Surgery and Microbiology and Immunology
Program Coordinator for Education
Cardinal Bernardin Cancer Center
Health Sciences Division
Loyola University Chicago, USA

Dr Stephanie Watkins obtained her PhD from the University of Louisville in 2007 for a project investigating the plasticity of tumour-associated macrophages. Here, she showed that anti-inflammatory tumour-associated macrophages become pro-inflammatory under IL-12 cytokine therapy and induce cytotoxic T cells to infiltrate lung tumours. She continued her work as a postdoc studying the relationship between cancer and inflammation and earned her track to tenure at Loyola University Chicago through a Pathway to Independence NIH grant. She is a member of the American Association of Immunologists (AAI), American Association for Cancer Research and several other oncology and immunology dedicated associations. Over the course of her career has received no less than 14 awards for her work, including a prestigious Cynthia Chamber-memorial Award from the AAI and a Research Scholar Grant from the American Cancer Society. In addition to her dedication to cancer research she is involved raising awareness through Public policy and community outreach programs to enhance science education in public schools.

CONTACT

E: swatkins1@luc.edu
T: (+1) 708 327 3361
W: https://www.researchgate.net/profile/Stephanie_Watkins
W: https://www.loyolamedicine.org/doctor/stephanie-watkins

LOYOLA UNIVERSITY CHICAGO

KEY COLLABORATORS

Dr Caroline Le Poole, Loyola University Chicago
Dr Jose Guevara-Patino, Loyola University Chicago
Dr Brian Baker, University of Notre Dame
Dr Elizabeth Kovacs, University of Colorado Denver

FUNDING

NIH
Loyola University Chicago
Trinity Health Systems
American Cancer Society

REFERENCES

MG Thompson, M Larson, A Vidrine, K Barrios, F Navarro, K Meyers, P Simms, K Prajapati, L Chitsike, LM Hellman, BM Baker and SK Watkins, FOXO3-NF-κB RelA Protein Complexes Reduce Proinflammatory Cell Signaling and Function, J. Immunol., 2015, 195, 5637–47.

SK Watkins, B Li, KS Richardson, K Head, NK Egilmez, Q Zeng, J Suttles and RD Stout, Rapid release of cytoplasmic IL-15 from tumor-associated macrophages is an initial and critical event in IL-12-initiated tumor regression, Eur. J. Immunol., 2009, 39, 2126–35.

SK Watkins, NK Egilmez, J Suttles and RD Stout, IL-12 rapidly alters the functional profile of tumor-associated and tumor-infiltrating macrophages in vitro and in vivo, J Immunol., 2007, 178, 1357–62.