Personal Note

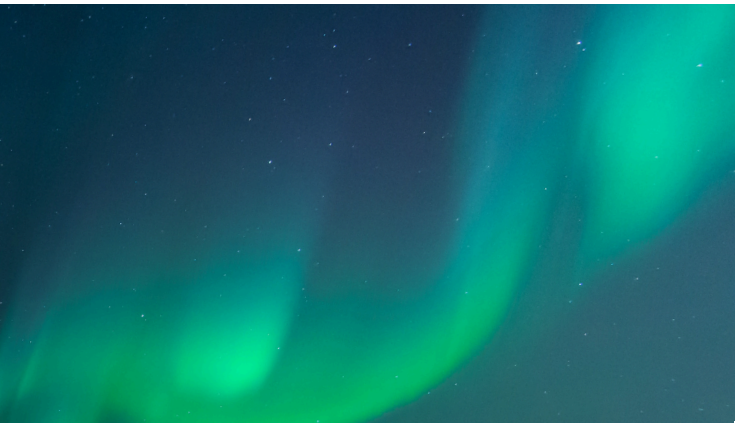
Achieving this work involved many late nights, weekends, and countless challenges. But I was not alone, and I could not have done it without the support of so many people.

Thank you to my supervisors Kaat, Lisa, and Bram for your guidance and encouragement throughout the years. I am also grateful to Frank, Emmy, and Gabe for their mentorship at different stages of this journey.

This work would not have been possible without the contribution of skilled chemists from the Laboratory of Medicinal Chemistry. Thank you Simon, Serge, and Anouk for the team effort. I am also grateful to the PPOL team, especially Fien, whose contributions were central to this work.

Thank you to all my friends, who I know I can always count on. A special thanks to the Fappy/Mappy group, and especially my paranymphs—Sarah-Lee, Lisa, Elien, and Iris. What originally began as colleagues, I am fortunate now to call you guys friends.

Finally, to my family, and especially my parents: 21 years ago you made the brave decision to move abroad for the sake of your children's education. This PhD is a testament to all the sacrifices you have made over the years. Thank you from the bottom of my heart.



Doctoral Advisory Committee

Prof. Dr. Katleen De Preter
Department of Biomolecular Medicine,
Ghent University, Belgium

Prof. Dr. Pieter Mestdagh
Department of Biomolecular Medicine,
Ghent University, Belgium

Examination Committee

Prof. Dr. Katleen De Preter (**chair**)
Department of Biomolecular Medicine,
Ghent University, Belgium

Prof. Dr. Steven Goossens
Department of Diagnostic Sciences,
Ghent University, Belgium

Prof. Dr. David Creytens (M.D.)
Department of Diagnostic Sciences,
Ghent University, Belgium

Dr. Benoit Louage
Department of Pharmaceutics,
Ghent University, Belgium

Prof. Dr. Adam Durbin (M.D.)
Department of Oncology,
St. Jude Children's Research Hospital, USA

Prof. Dr. Jan Molenaar (M.D.)
Group leader Molenaar team,
Princess Máxima Center for Pediatric Oncology,
The Netherlands

Host Laboratory

Pediatric Precision Oncology Lab
Department of Biomolecular Medicine
Ghent University, Belgium

GHENT UNIVERSITY

FACULTY OF MEDICINE AND HEALTH SCIENCES

CHASING AURORA

HARNESSING TARGETED PROTEIN DEGRADATION OF AURORA KINASE A AS A NOVEL THERAPEUTIC MODALITY IN NEUROBLASTOMA

Muhammad Rishfi (Affy)

A dissertation submitted to obtain the degree of Doctor of Health Sciences

27 October 2025

Promotor
Prof. Dr. Kaat Durinck

Co-promotors
Dr. Lisa Depestel
Prof. Dr. Bram De Wilde (M.D.)

Summary

Today, many **children with cancer** still receive treatments originally developed for adults. But children are not just "miniature adults." Their cancers develop through **unique** biological processes, which means they require dedicated research and tailored therapies.

Neuroblastoma, a cancer of the sympathetic nervous system, is one of the deadliest childhood cancers, responsible for about 15% of all childhood cancer deaths. Current **treatments are highly intensive** and **often not enough.** Only half of children with high-risk neuroblastoma survive, and those who do, often suffer serious **long-term side effects** from the harsh therapies. There is an urgent need to develop safer and more effective therapies for this deadly disease.

This work explores new ways to treat neuroblastoma by targeting a protein called **Aurora Kinase A (AURKA)**. We developed a relatively new class of drugs, called **PROTACs**, that can completely remove/degrade this protein from cancer cells. Our **first-generation** compound, **PROTAC SK2188,** was able to **slow the growth** of neuroblastoma cells in the lab and even trigger them to undergo **cell death**. This approach also showed promise in removing another and very important cancer-driving protein, MYCN – although this effect occurred more slowly and requires further research (Manuscript 1).

Before any clinical trials in humans can be done, potential drugs must be tested in animal models to assess their safety and efficacy. However, due to its bulky chemical structure**, SK2188 was unstable** and broke down too quickly after administration in mice. To overcome this, we created **second-generation** compounds, **PROTAC SK4454** and **SK5527.** These have more drug-like structures and show **improved stability**, making them more suitable for testing in animal models – an essential step before considering testing in patients (Manuscript 2).

Importantly, even with protein-degrading drugs like PROTACs, cancer cells are often **resistant to treatment with a single drug**. For this reason, cancers are usually treated with **combinations of different drugs**. To explore this, we carried out a large-scale drug screen to see **which drugs could be effective when combined with our PROTACs**. Our preliminary findings show that combining our PROTACs with the compounds **Volasertib** (which blocks a protein called PLK1), or **Tipifarnib** (which blocks a protein called FNTB) show powerful synergistic effects. Further research is now needed to understand why these combinations are effective and how they can be developed into future therapies for children with neuroblastoma (Manuscript 3).

An online version of the thesis and this leaflet can be found here

SCAN ME

Publications Included in this work

Manuscript 1: Targeted AURKA degradation: Towards new therapeutic agents in neuroblastoma.
Muhammad Rishfi & Simon Krols et al.,
European Journal of Medicinal Chemistry (2023)

Manuscript 2: Second-generation AURKA-targeting PROTACs: Structural optimization towards *in vivo* degradation in neuroblastoma.
Muhammad Rishfi & Simon Krols et al.,
Journal of Medicinal Chemistry (under review)

Manuscript 3: Combination drug screening and proteomics analysis reveal PLK1 and FNTB as synergistic drug targets with AURKA in neuroblastoma.
Muhammad Rishfi et al.,
In preparation

Patents

Aurora kinase A Degraders (EP25185905.4)
filed on 27/06/25

About the author

Ph.D. in Health Sciences Ghent University, Belgium	2020 - 2025
Ph.D. Research stay abroad Children's Cancer Institute, Australia	2023
B. Sc., & M. Sc., in Biomedical Sciences Ghent University, Belgium	2014 - 2020
Preparatory Language Year (Dutch) KU Leuven, Belgium	2013 - 2014
Primary & Secondary Schools International School of Brussels, Belgium American School of Barcelona, Spain SDI Sudirman Jakarta, Indonesia	2001 - 2013

THIS RESEARCH WAS SPONSORED BY

fwo · Villa Joep! · Kom op tegen Kanker · stichting tegen kanker