PUBLICATIONS INCLUDED IN THE THESIS

Paper 1
Marcos Rubio, A. et al. Circulating immune cell dynamics as outcome predictors for immunotherapy in non-small cell lung cancer. *Journal for ImmunoTherapy of Cancer* (2023).

Paper 2
Marcos Rubio, A. et al. Multi-omics dataset of peripheral blood immune cells from lung cancer patients treated with immunotherapy. *To be submitted in 2025.*

Paper 3
Marcos Rubio, A. et al. Defining the optimal setting for transcriptomic analyses on blood samples for response prediction in immunotherapy-treated NSCLC patients. *Scientific Reports* (2024).

CONTRIBUTED TO
Everaert, C., Verwilt, J., et al. Blocking abundant RNA transcripts by high-affinity oligonucleotides during transcriptome library preparation. *Biological Procedures Online* (2023).

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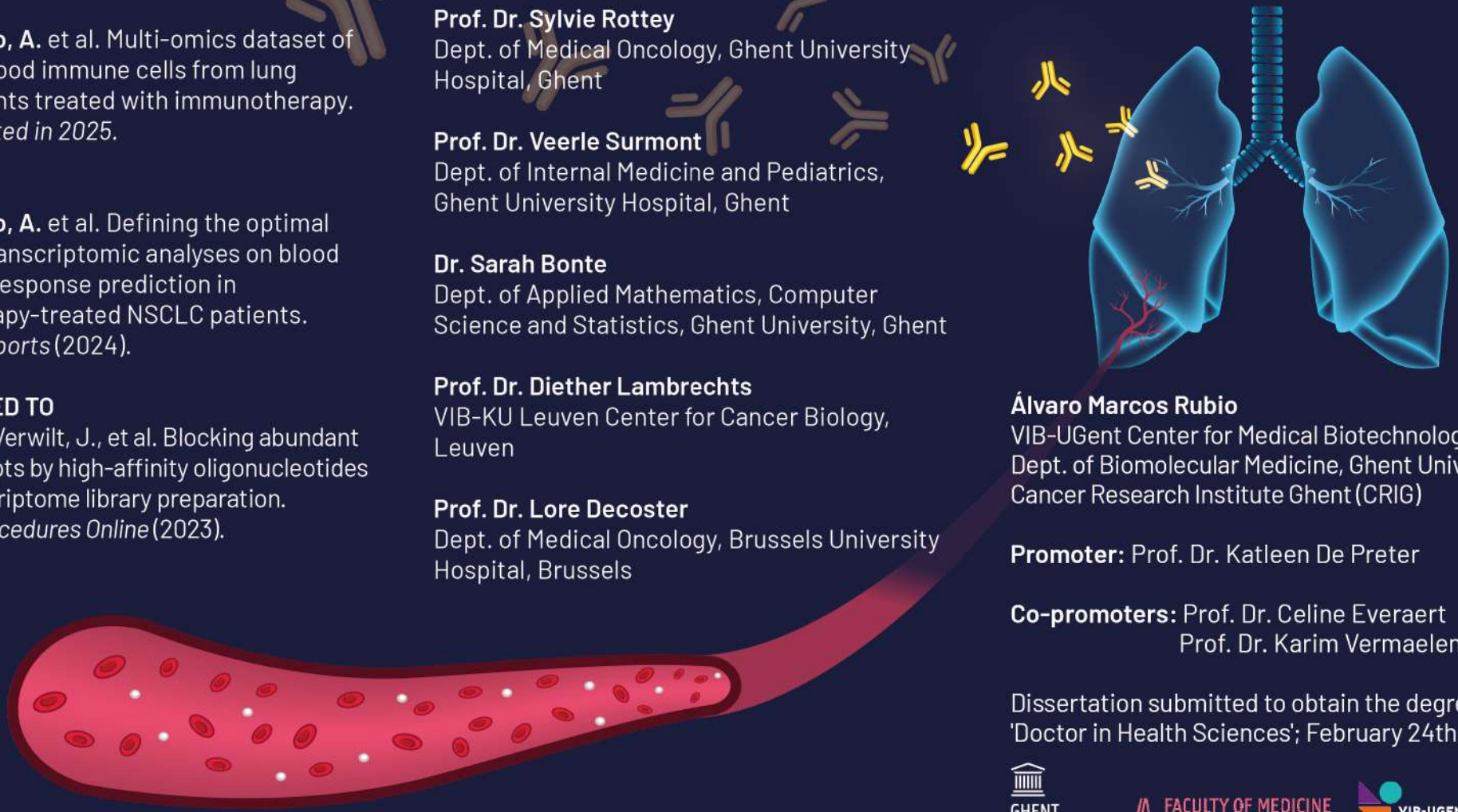
Systemic immune epigenomic predictors of immunotherapy response in NSCLC patients: Potential and pitfalls



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When a tumor appears in the body, nearby immune cells recognize it as a malignant entity and attack the tumor cells through a process called the "cancer-immunity cycle". However, this cycle is often blocked due to the expression of immune checkpoint molecules by the tumor cells. These molecules stop immune cells from killing cancer cells so that the tumor can continue growing. In the last decade, **a new therapeutic strategy, called immune checkpoint inhibitors (ICIs)**, has been developed to restore the immune activity against the tumor that was blocked by immune checkpoint molecules. Due to its great success in eliminating cancer cells from the body and extending the life of many patients, it is now one of the main treatment options for several cancer types, including **non-small cell lung cancer (NSCLC)**.

Nevertheless, **only a small fraction of NSCLC patients (<30%) respond to ICIs**, which means that the majority of the patients lose valuable time being treated with a therapy that will not be beneficial for them. Moreover, ICIs are a very expensive treatment for the public health care system, and some patients can develop serious side effects. This emphasizes the importance of **predicting which patients will and will not benefit from an ICI treatment**. As of now, three predictive tests (also called biomarkers) can be used in the clinic to distinguish between responder and non-responder NSCLC patients before ICI therapy. However, these biomarkers are not totally accurate, and require the collection of a piece of the tumor through a surgical procedure, which is uncomfortable and can even be dangerous for some patients. On the contrary, **non-invasive biomarkers offer a more patient-friendly alternative**. Recently, some immune cell types found in the blood of NSCLC patients have been associated with a good response to ICIs, using a method called flow cytometry. However, this technique requires pre-defined cellular markers, limiting the amount of immune cell types that can be characterized. By **studying the epigenomics** (the processes that determine which genes are expressed in each cell and define its identity) **of the immune cells using next generation sequencing (NGS), we can characterize them** in a more unbiased way. Ideally, we would do this one single cell at a time, but this would be too expensive to be implemented in a clinical setting. Bulk sequencing, however, can provide a more affordable approach to characterize the systemic immunity of NSCLC patients before ICI treatment.

Therefore, this thesis addresses the question: **What is the most appropriate analytical and computational approach to identify systemic immune bulk epigenomic biomarkers that predict ICI response in NSCLC patients?**

RESULTS

Considering all the studies investigating systemic immune cells as predictors of immunotherapy response in NSCLC, **the most predictive ones are CD8+ and CD4+ T cells, and the neutrophil to lymphocyte ratio (paper 1)**. These markers are measured using flow cytometry or manual cell counts. To investigate the potential of bulk NGS to identify systemic immune epigenomic biomarkers for ICI response prediction, **we generated RNA expression, chromatin accessibility, and DNA methylation profiles of peripheral blood mononuclear cells (PBMCs) and whole blood (WB) samples from 33 ICI-treated NSCLC patients**. Quality assessment confirmed the high integrity of this data, establishing a valuable resource for further research (**paper 2**). Using the RNA expression data, We evaluated the best settings for distinguishing responders from non-responders by comparing three variables: sample type (PBMCs vs. WB), sample collection timepoint (baseline vs. post-treatment), and computational method (differential gene expression analysis, gene set enrichment analysis, cell type deconvolution). Results indicated that **post-treatment WB samples and gene set enrichment analysis provided the most accurate insights (paper 3)**. To further explore the predictive potential of chromatin accessibility and DNA methylation data, we applied the same computational methods as in the RNA expression analysis. Preliminary findings from gene set enrichment analysis suggested that **circulating granulocytes and inflammatory response pathways may negatively impact ICI response**, while immune cell deconvolution showed a non-significant trend of **higher granulocyte proportions in non-responders.**

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