Understanding the Role of Regulatory T Cells in Breast Cancer Metastasis

Breast cancer is the most common cancer amongst women accounting for 24 percent of new cancer cases worldwide and 15 percent of cancer deaths in 2018.[1] And cases are expected to increase by more than 46 percent by 2040, according to a recent article in The Lancet.[2]

While survival rates for breast cancer patients are improving with the help of early detection, the incurable metastatic stage of the disease has a poor prognosis, resulting in most breast cancer deaths. Of the 1.7 million new cases of breast cancer diagnosed annually worldwide, around 30 percent of patients diagnosed with localised disease get metastases in distant organs.[3]

How the immune system helps or hinders metastases is a promising field of breast cancer research. In the micro-environment in the tumour, for example, tumour-associated macrophages (TAMs) are associated with invasion, metastasis, and a worse prognosis. But tumour-infiltrating lymphocytes (TILs) are associated with a better outcome.

Cancers host a plethora of other immune cell subsets too, such as lymphocytes, various myeloid cells, and innate lymphoid cells, some of which aid and abet the tumour, while others hinder its progress.

Kevin Kos, a molecular immunologist in Karin de Visser's Inflammation and Cancer research group at the Netherlands Cancer Institute (NKI)- in Amsterdam, and researcher of Oncode Institute, has been using Qlucore's Omics Explorer for his PhD research on the role of T regulatory cells (Tregs) in breast cancer metastasis.

"Tregs act like the military policemen among the white blood cell population. Whereas other white blood cells fight pathogens, Tregs regulate immune responses to ensure that nothing goes out of control, making sure there isn't excessive inflammation (Kos and de Visser, 2021). When this goes wrong, such as in auto-immune diseases like diabetes, multiple sclerosis, and inflammatory bowel disease, Tregs are either dysfunctional or reduced," Kos explains.

The Netherlands Cancer Institute, founded in 1913, is a comprehensive cancer centre with a hospital and research lab. The lab is staffed by 750 scientists and scientific support personnel, while the hospital has 230 medical specialists, 212 beds, and an out-patients clinic with around 140.000 visits annually.

"There are many different projects running in our group," says Kos. "On the clinical side, for example, we analyse patient samples to understand how various immune parameters change in breast cancer patients. I'm focused on the pre-clinical work, looking at how cancer spreads throughout the body and how the immune system is involved."

To track the complex process of metastasis development, Kos is using mouse models in a way that closely mimics cancer development in humans.

"We use mice that are genetically engineered to develop tumours in their mammary glands. When these mice are about 8 months old, the tumours start to become palpable. At that point, we can test treatments such as chemotherapies and different combinations of treatments," he explains.

"Additionally, we can also put small tumour fragments into healthy mice. When these tumours grow, we can surgically remove them. But like in human patients, these tumours recur in distant organs in the form of metastases. Within this window of disease progression, we can test novel treatments or other forms of analyses to study metastasis development."

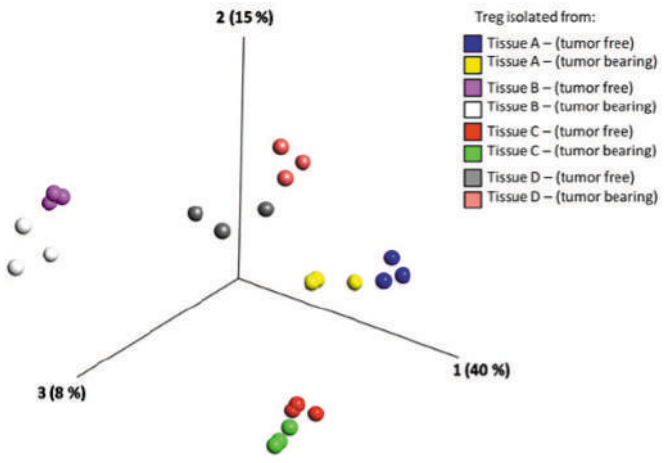
Kos is using Qlucore's Omics Explorer to perform bulk RNA sequencing analysis on isolated Treg cells from various tissues such as the blood and lungs from both tumour-bearing and healthy mice.

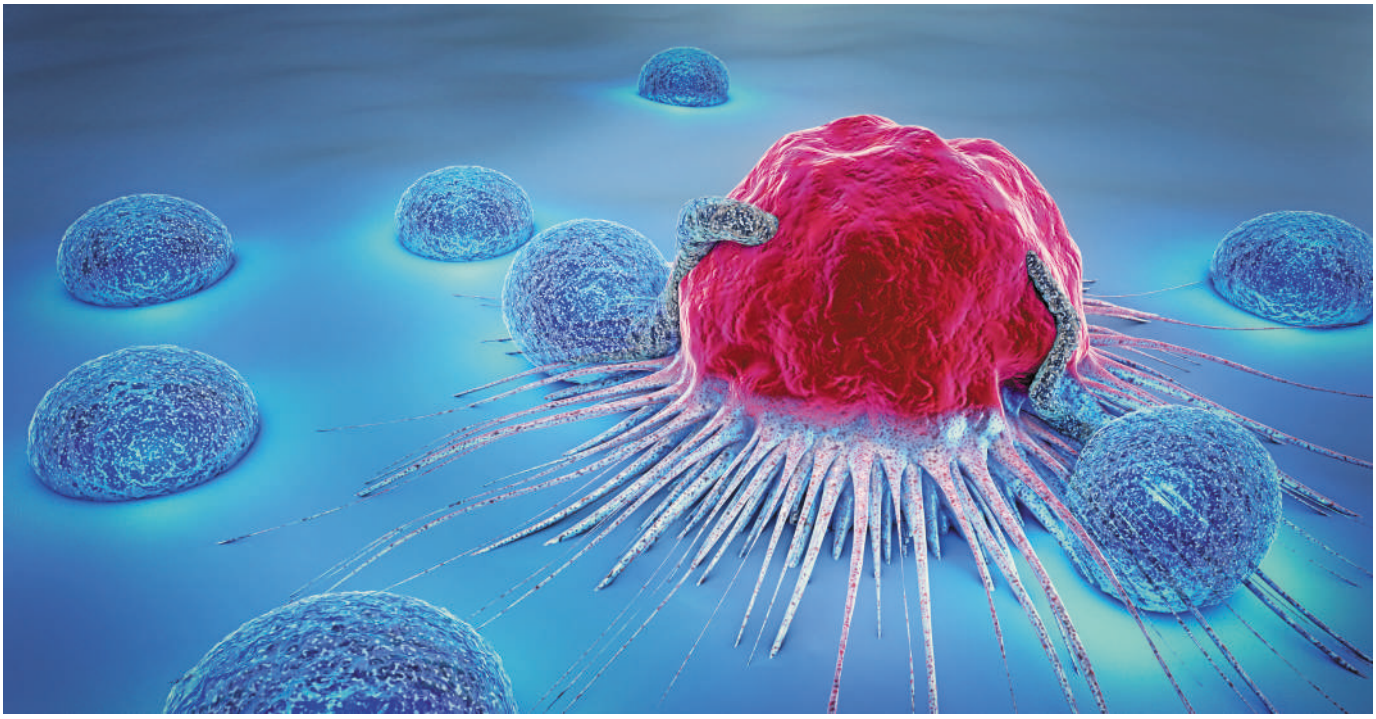
"By doing this, we can see what kinds of mRNA the Treg cells express, which is a surrogate for what kind proteins they are making. So, we're finding out whether they are active, are they dividing, are they functional. We learn all this from sequencing data," he explains.

"Tumours affect the immune system throughout the body. In human cancer patients, their blood is very different from the blood of healthy patients," says Kos. "Ultimately, we want to use these insights to develop new interventions that may halt tumour progression."

Easy Analysis and Visualisation of Transcriptional Data

The Qlucore tool is used to perform differential gene expression analysis, clustering analysis, PCA analysis, and to produce publication quality figures of the transcriptome analyses.





Differential gene expression analysis (see above) has been particularly useful in this project. "You get a statistical value for the differences between big groups," says Kos. "We are sequencing thousands of different transcripts and to make sense of that it's vital to see which genes are truly different between the subjects we're comparing, such as healthy versus tumour-bearing mice."

As with many research institutes, the bioinformaticians at the NKI have their own projects and very little free time to assist on ones that aren't related. "The main benefit of using Qlucore is that it bypasses this need, and allows for easy, do-it-yourself, in-depth analysis of complex RNAseq data," says Kos.

The first paper from this research is under review and due to be published soon. Initial findings suggest that in all tissues analysed, Tregs behave differently in tumour-bearing mice compared to healthy mice.

Kos says: "It probably happens for more immune cells, not just Tregs. What we didn't expect was that cells would be affected in distant organs like the spleen when the primary tumour is in the mammary glands. It's a bit of a shocker. It means that the tumour is influencing Tregs far beyond their local environment. This behaviour has already been described for myeloid cells, so it's interesting to observe it in lymphocytes as well," he adds.

The results of this research may help to better understand the interaction between the immune system and breast cancer.

"Ultimately, the idea is to stop the Treg action for a short time in a way that interferes with functions that might help tumours to grow," summarises Kos.

Ready to Handle More Complex Single Cell Sequencing Data

Bioinformatics analyses are getting increasingly complex. While this latest project is about bulk RNA sequencing of thousands of cells, the next step is to look at the gene expression in individual cells. Other scientists in the lab are doing that with patient samples.

"This is the future for gaining a much deeper understanding. You can see what is different between a specific patient and a healthy person," says Kos. "These kinds of analysis can also be done with the Qlucore tool. It's super-nice as these kinds of analysis can normally not be performed by researchers that do not have the experience of bioinformaticians."

REFERENCES

1. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries by H Sung et al; CA Cancer J Clin. 2021
2. Global burden and trends in premenopausal and postmenopausal breast cancer: a population-based study by Emily Heer et al; The Lancet August, 2020
3. The genomic landscape of metastasis in treatment-naïve breast cancer models by Christina Ross et al; PLoS Genet, May 28, 2020
4. Kos, K., de Visser, K.E., 2021. The Multifaceted Role of Regulatory T Cells in Breast Cancer. Annu. Rev. Cancer Biol. 5. https://doi.org/10.1146/annurev-cancerbio-042920-104912

Christine Evans-Pughe

Christine Evans-Pughe is a freelance science and technology writer whose work has featured in The Economist, The Guardian, The Independent, and BBC Focus as well as a wide variety of specialist journals. She was a regular feature writer for the UK's Institution of Engineering & Technology's award-winning Engineering & Technology magazine for over 15 years, and is co-founder of the website www.howandwhy.com.

Email: qlucoreinfo@qlucore.com