# **Deciphering the Signaling Network Landscape of Breast Cancer to Enable Personalized Medicine**

# **Introduction**

Breast cancer is the **most common cancer in women** worldwide1 and the second most common cause of cancer related death in women.2 Breast cancer is highly heterogeneous and is divided into different subtypes with distinct molecular features and clinical behaviors.3 Not all women within the same clinical subtype respond to targeted therapy and even those who do respond often present resistance during or reoccurrence after therapy. The most aggressive subtypes such as triple negative breast cancer lack a targeted therapy. While **breast cancer variability at the genomic and transcriptomic levels is well characterized,**4-7 **little is known about its effect on the variability of the signaling biology** (*i.e.* network wiring), and how the cells respond to environmental influences such a drug treatment.

Knowledge on how genomic aberrations affect the signaling network structure and in turn how drug perturbations of kinases change the signaling network state are key to deepen our understanding of signaling biology and cancer biology. Ultimately, this knowledge will pave the way to a genomic based drug selection to achieve high efficacy and effectiveness. This knowledge gap is mostly due to a technological gap as **absolute quantities at the single-cell level would be required in order to understand the network structure**. Absolute quantities are needed as to obtain the relative stoichiometries of the network nodes. Single-cell measurements enable the application of computational tools to identify causal relationships between network nodes, something not feasible with classical “population” measurements (*e.g.* proteomic and transcriptomic) as cells responding differentially are averaged together. In this project I will lay the foundation to relate cancer signaling biology to genotypes and perturbation using the concrete example of breast cancer with an innovative experimental setup.

## Aim

The overall goal of this project is to **determine how genome alterations shape signaling network biology and the signaling response to perturbation such as kinase inhibitors.**

# Results and Outlook

## Signaling heterogeneity

The aim is to analyze a breast cancer panel of cell lines (67) that **covers all the commonly found genomic alterations** in cancer and the transcriptomic and genomic profiles used by clinical pathologists.8-13

To understand how the signaling network response differs depending on the genomic background, we measured all cell lines under 46 conditions. The applied conditions include: (i) ten **EGF stimulation** time points ranging between zero and one hour; (ii) growth in full medium; and (iii) seven EGF stimulation time points in the presence of **well-established inhibitors of five important kinase pathways** (PI3K, mTOR, EGFR, PKC, MEK). All the samples were characterized with an antibody panel covering a **37-nodes network (mostly phosphoproteins)**. Furthermore, relevant samples will be analyzed with a panel focusing on the levels of biochemically and clinically significant markers as well as by **data-independent acquisition mass spectrometry**.

During my first three years I **established the cell line bank** (over 1500 frozen aliquots), I fine-tuned the setting and collection methods for the characterization of breast cancer heterogeneity, and I **collected all the 3944 samples for mass-cytometry** and **201 samples for proteomics**. Furthermore, all cell lines have been **already measured by mass cytometry** and by mass spectroscopy. Finally, the data analysis in **collaboration with Prof. Andreas Beyer** (University of Cologne)has started. With the obtained data I could already appreciate **cell line specific phenotypes and how known genetic perturbations affect the signaling network structure**. I also could see some expected behaviors already described in the literature.

The plan now is to **analyze four of the already measured cell lines from a specific clinical background** (triple negative) along with two hormone positive one. Then to wrap up these data in a first, **more-descriptive and non-genotype centered paper**. Meanwhile on the background I will keep working to finalize the measurement and analysis of the larger screen.

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