Molecular characterisation of drug-tolerant persister cells to overcome chemotherapy induced relapse in breast cancer

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Background

The majority of primary **breast cancers** respond to **chemotherapeutic intervention**. However, years or even decades later many women suffer from **relapse**, which **gradually lose drug sensitivity** until ultimately **resistance develops**.

To prevent these, we aim **to investigate the few cancer cells,** which **withstand initial chemotherapy**, without being resistant a priori. These cells are called "**drug tolerant persister cells**" (DTPs).

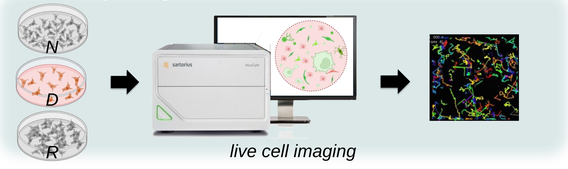
Hypothesis

Chemotherapeutic drug treatment bequeaths a rare surviving **quiescent persister cell population,** which represents a **lurking reservoir of surviving cells**. These cells eventually give rise to **relapsing tumours** and potentially constitute the **causative factor for drug resistance**.

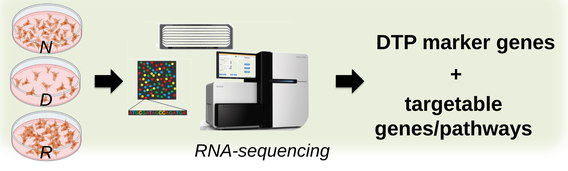
Aims

AIM 1: *In vivo* identification and tracking of DTPs

AIM 2: Morphological characterisation of DTPs



AIM 3: Molecular and functional characterisation of DTPs



KB1P…breast cancer organoid, mCA…mCherry/Akaluc plasmid, BLI…biolum. imaging, N= naïve, D= DTP, R= repopulated

Results

AIM 1:

The successful generation of a **polycistronic lentiviral construct** expressing the **AkaLuc luciferase** and the **fluorescent reporter protein mCherry (mCA)** allowed the transduction of **murine breast cancer organoids (KB1P)** resulting in the **mCA-KB1P** model for the **non-invasive intravital detection and tracking of rare DTP** cells in living mice.

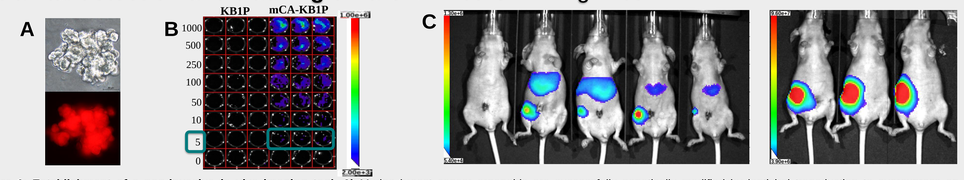


Figure 1: Establishment of a non-invasive *in vivo* imaging tool. A) Murine breast cancer organoids are successfully genetically modified by lentiviral transduction to overexpress an mCherry-AkaLuc reporter. Bottom panel shows KB1P organoids strongly expressing the fluorescent reporter, mCherry. **B)** *In vitro* AkaBLI detects ~ 5 cells in a plate based titration assay. **C)** Engrafted mCA-KB1P organoids retain their BLI signal. Left panel shows a control KB1P-transplanted mouse and four mCA-KB1P4 transplanted mice 48 hrs after mammary fat pad transplantation of $5x10^4$ cells. The liver signal is an unspecific signal of the substrate. Right panel shows three mCA-KB1P-transplanted mice at day 44 after transplantation.

AIM 2:

Live cell imaging pilot studies of the DTP cell phase showed **dynamic changes** of the **motility** and **morphology** of DTPs and recorded their **reawakening after a dormancy** period of 15 days. We generated a cell line with fluorescently labelled nuclei and cytoplasm, which enables *in vitro* DTP **tracking** and **cell size** determination of DTP cells. This will allow the **morphological characterisation** and **classification** of different DTP cell **subpopulations**.

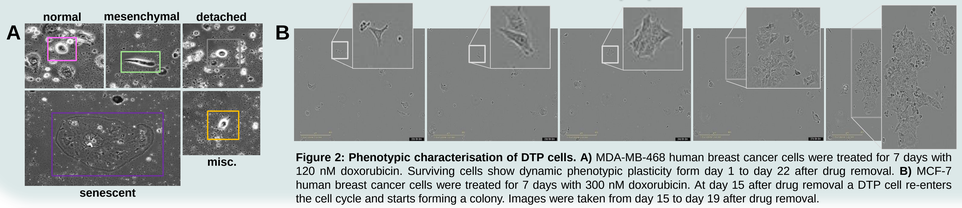


Figure 2: Phenotypic characterisation of DTP cells. A) MDA-MB-468 human breast cancer cells were treated for 7 days with 120 nM doxorubicin. Surviving cells show dynamic phenotypic plasticity form day 1 to day 22 after drug removal. **B)** MCF-7 human breast cancer cells were treated for 7 days with 300 nM doxorubicin. At day 15 after drug removal a DTP cell re-enters the cell cycle and starts forming a colony. Images were taken from day 15 to day 19 after drug removal.

AIM 3:

RNA-sequencing of drug-naïve, DTP and repopulated cells shows the distinctness of the three populations, suggests **subpopulations within the DTP cell pool** and reveals a transcriptional **DTP signature.** We validated selected **DTP marker genes** which will be used to investigate the fate of the DTP cells both *in vitro* and *in vivo*. The determination of potential **key regulators of the DTP cell phase** will help to identify promising **drug targets**.

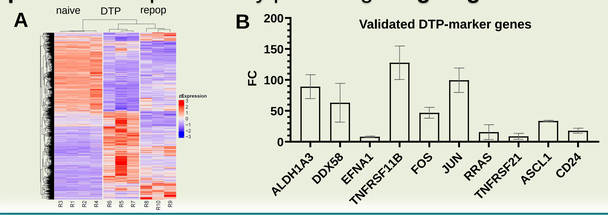


Figure 3: Molecular characterisation of DTP cells. A) RNAseq reveals distinct transcriptional profiles for DTP and repopulated cells. Up-regulated genes are depicted in red and down-regulated genes are shown in blue. Noteworthy, subpopulations within the DTP cell pool can be determined. **B)** Selected *in vitro* validated DTP-marker genes show substantial upregulation in the DTP cell phase compared to the untreated, naïve cells. Fold change (FC) values of the DTP cell phase are shown for each gene.

Conclusions

Summarising, we have established a **non-invasive *in vivo* imaging tool,** which will be used to **identify and track DTP cells** in living animals.

Moreover, our **live cell imaging** approach revealed the plasticity of the surviving cells after chemotherapy. This will allow **sub-classification of DTP cells** to identify cells with the potential of re-entering the cell cycle and thereby giving rise to proliferating colonies.

The determination of a **DTP cell specific transcriptional signature** will help to identify **potential drug targets** and will contribute to **unveil the Achilles' heel** of the few surviving DTP cells to eventually **overcome chemotherapy induced relapse in breast cancer.**

Literature cited

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Further sources

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