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MammaExplant: Development of a human breast cancer explant model to replace murine tumor models

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Background

Tumor-associated macrophages (TAMs) play an important role in the tumor microenvironment by promoting tumor progression and immunosuppression. In breast cancer, TAMs can account for a considerable proportion of the tumor mass, and a detailed understanding of their interaction with the surrounding cancer cells could enable better cancer therapies in the long term. However, such experiments cannot be done in cell culture, but must be studied in animal models or better directly in the human system. The aim of our 3R project is therefore to develop a breast tumor explant model that can systematically **replace** corresponding animal experiments and be used for cancer drug screening and optimization.

Methods

RWTH cBMB, the Biobank of the Medical Faculty at RWTH Aachen University, has successfully established the logistics for delivering freshly prepared breast tumor tissue to its cooperation partner Navitect Bio in Heidelberg within a few hours after surgery. At Navitect Bio, the tissue is cultivated immediately using established methods and optimized media. The novel synthetic antibodies provided by Syntab Therapeutics, known as Immune System Engagers (ISErs), are then tested for their effectiveness in activating the anti-tumor immunity of TAMs and other immune cells.

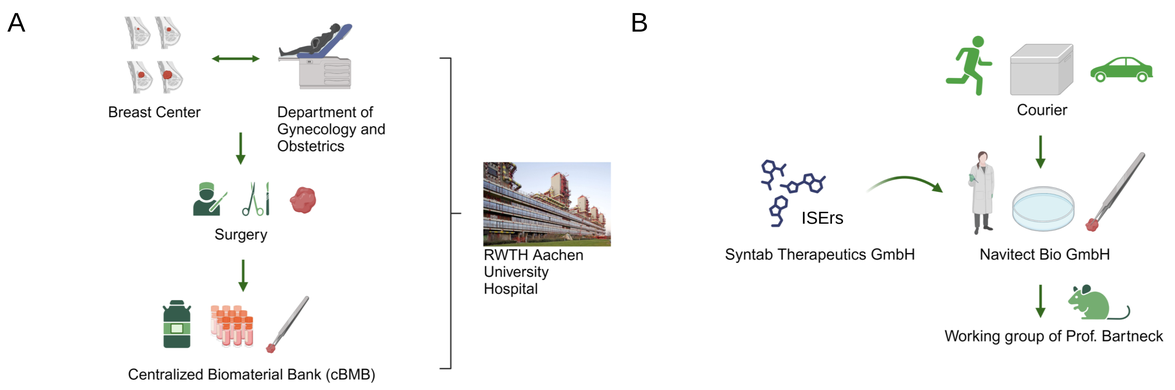


Figure 1. Route of administration of breast cancer tissue. A: Procedure within RWTH University Hospital B: Shipment of the sample from Aachen to the project partner Navitect Bio in Heidelberg, followed by returning the sample to Aachen.

Results

Using the explant shipment sequence described above, we were able to culture the breast cancer explants for six days and it was successfully established. Navitect Bio performed immunohistochemical analyses of the tissues and investigated several relevant surface markers of various immune cells as well as the proliferation and apoptosis of immune and tumor cells. The Bartneck group at UKA is using state-of-the-art techniques such as flow cytometry and OMICS technologies such as RNA sequencing or proteomics to identify key factors for the evaluation of the anti-tumor response. Initial experiments with ISErs have also been carried out, which look promising, but of course need to be confirmed in further experiments.

1. The breast cancer explant

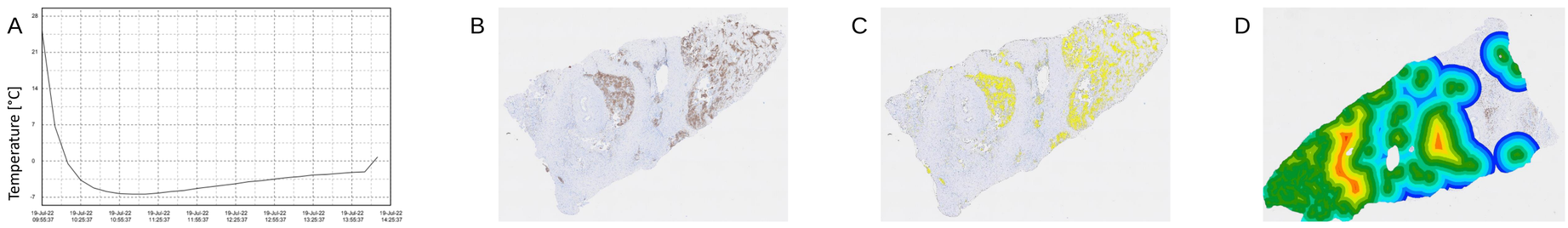


Figure 1. Establishment of the breast cancer explant model. A: Temperature profile displaying the tracked temperature obtained from a temperature logger during the transport of the fresh tumor tissue from Aachen to Heidelberg. B: Immunohistochemical (IHC) staining of tumor marker EpCAM on day 0. C: Tumor mapping based on Fig.1B utilizing the image analysis platform HALO AI (Indica Labs) with integrated machine learning. D: Spatial definition for infiltration analysis after tumor mapping based on 1B.

2. Enrichment of CD3+ T cells in tumor zones after ISEr administration

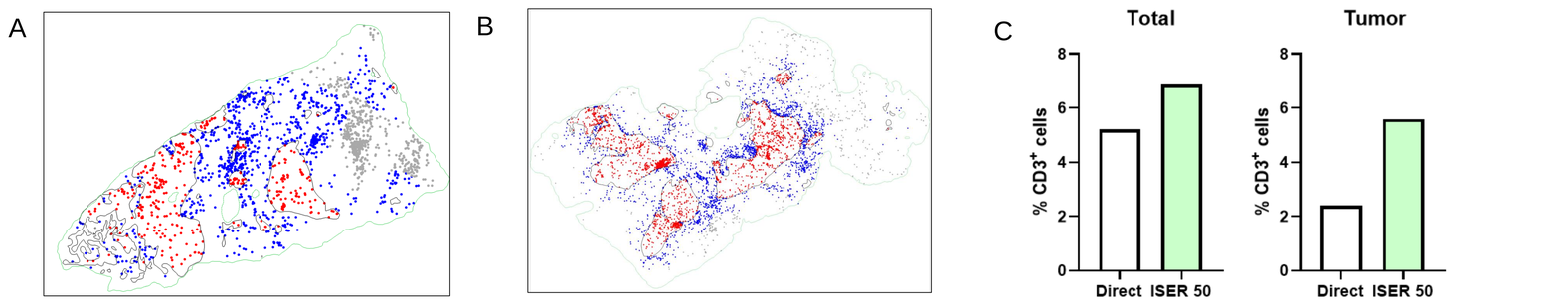


Figure 2. Tumor mapping depicting distinct cell populations. Tumor tissue was marked with black line within the entire tissue piece. A: Distribution of the CD3+ cells on day 0 of the cultivation, showing CD3+ colonies in normal tissue (extratumoral) marked with blue color and intratumoral, marked with red. B: Distribution of the CD3+ cells after addition of ISEr (50 nM) on day 2 of the cultivation. C: Graphs representing CD3+ cells in total tissue slice (left graph) and in tumor tissue only (right graph) after addition of ISEr (50 nM) on day 2 of the cultivation.

3. Depletion of CD163+ Tumor-associated macrophages in tumor zones after ISEr administration

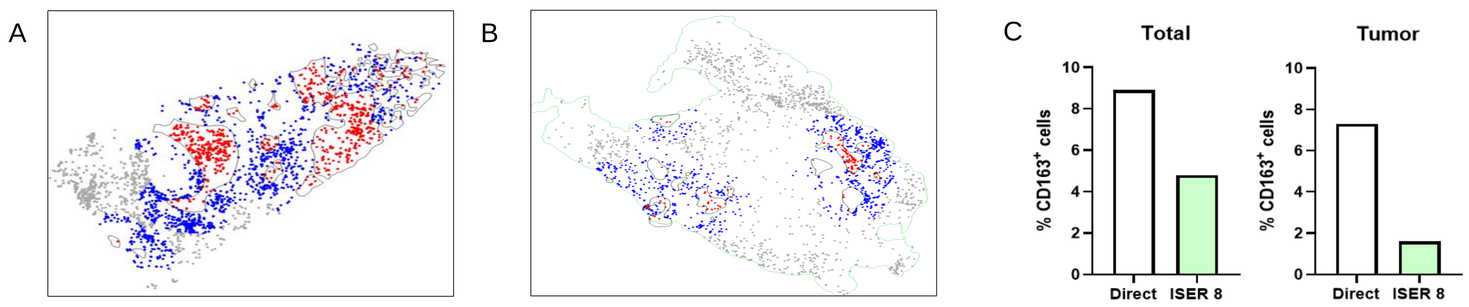
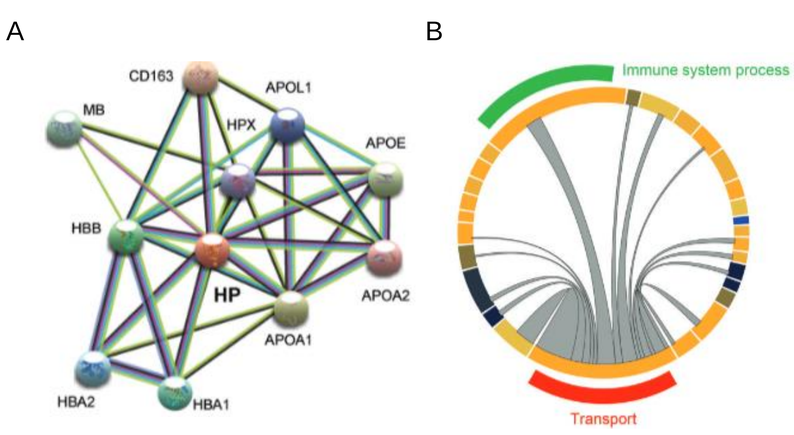


Figure 3. Tumor mapping depicting distinct cell populations. Tumor tissue was marked with black line within the entire tissue piece. A: Distribution of the CD163+ cells on day 0 of the cultivation, showing CD163+ colonies in normal tissue (extratumoral) marked with blue color and intratumoral, marked with red. B: Distribution of the CD163+ cells after addition of ISEr (8 nM) on day 2 of the cultivation. C: Graphs representing CD163+ cells in total tissue slice (left graph) and in the tumor only (right graph) after addition of ISEr (8 nM) on day 2 of the cultivation.

4. Proteomics



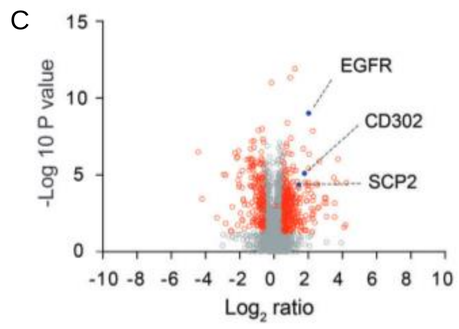


Figure 4. Readout for the upcoming Proteomics analysis. A: Functional protein associatiation analysis. B: Enrichment analysis of upregulated proteins. C: Volcano plot analysis. Data from previous publication of AG Bartneck, Lin *et al.* (2023).

Outlook

In the MammaExplant project, we expect to gain new insights into the interaction of immune and tumor cells in the explant model and initial data on the efficacy of immunomodulatory ISEr drug candidates for later clinical application. In addition, by comparing the data obtained from the tumor explants to established murine tumor models, we aim to provide the basis for replacing mouse models with fresh, patient-derived human cancer explants, that display a fully intact organotypic tumor microenvironment and have the potential to support personalization of tumor therapy.