Therapy-induced priming of natural killer cells predicts patient-specific tumor rejection in multiple breast cancer indications

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ABSTRACT
Background: It is now clear that response or resistance to therapy reflects the tumor microenvironment, which is comprised of malignant cells, normal stroma, and immune landscape and is known to be the limiting factor to examine individual patient responses. In this study, we evaluate the tumor microenvironment, immune checkpoint inhibitors, which reestablish the body’s own immune defense, modulating lymphocyte activity including T cells and natural killer (NK) cells. However, clinical response is highly variable. There is a gap in our understanding for methods to predict clinical response or resistance to conventional and immunotherapies at the individual patient level.

Methods: Here, we used in vitro- and in vivo-experiments to study the role of NK cells in models of drug tolerance. Next, we employed CA(N)script, a clinically-validated ex-vivo tumor model that recreates and preserves the native patient tumor microenvironment, which integrates an algorithm-driven method to predict clinical response to therapy (M-Score). Utilizing tissue from patients diagnosed with luminal, HER2+, triple-negative, and triple-negative (KR- FP- HER2+) breast cancers, we studied spatial heterogeneity of CD56+ lymphocytes (NK) in the tumor stroma, M-Score, and alterations under control conditions or during pressure of conventional chemotherapy and immune checkpoint blockade. Furthermore, we investigated localization of other T-cell subsets and alterations to the cytokine profile (i.e., CCL6, NGF, class IIA/ B2M, MHC2, CD6, CD9, PanIK). Inflammation and pro-inflammatory cytokines.

Results: We identified that drug resistant breast cancer cells diminish the antitumor activity of NK cells. Interestingly, these correlations were evident with induction of the tumor-expressing biomarker CD56+NK cells, which is known to attract and recruit active NK cells. Using CA(N)script, we determined that a predicted response, determined by M-Score associated to increased presence of NK cells in tumors strains following drug pressure, which correlated broadly to a pro-inflammatory cytokine signature from the tumor microenvironment. These evidence were confirmed using multiple different conventional therapies for distinct breast cancer subtypes, and experimental immunotherapy agents.

Conclusions: Taken together, these data demonstrate an integral role that NK cells contribute to the antitumor effect of therapy including conventional and immune-modulatory drugs. It further demonstrates how a novel ex vivo platform can be developed to classify mechanisms of resistance and sensitivity, which couldn’t otherwise be known in a drug naïve state. Such an advance in our preclinical methods to study antitumor drugs at the individual patient level can help guide treatment decisions for clinicians while simultaneously functioning as a platform to study clinical efficacy of novel and emerging agents.

Fig. 1. Natural killer (NK) cell activity directly associates response or resistance to therapy in breast cancer models. Schematic depicting therapeutic responses on NK cells (MDCAB). A) NK cells conjugate ligands expressed on cancer cells (MICA/B). B) In-vivo coculture model: exposing NK cells to drug naïve or drug resistant Th1-cells is followed by flow cytometry for NK cell function. C) NK cell expression of activating biomarkers following co-culture with Th1 cells. D) In-vivo model: representative survival curves for different therapeutic groups (TH1 or R2). E) Tumor burden growth curve in the presence of NK cells or a NK-deficient mouse. F) Immunohistochemistry of tumors on day 16 tested for expression of MICA/B and indication of apoptosis (TUNEL).

Fig. 2 CA(N)script™, a multi-dimensional live tissue platform to predict patient-specific response to anticancer therapy. A) Ex vivo live tissue assay reliably recreates a patient’s native tumor ecosystem including microenvironment components, stroma, and immune components. B) Multi-dimensional quantitative interrogation performed indications of cell death, proliferation by IHC and cytokines as well as energy consumption by soluble analysis. C) The quantitative results from the multi-dimensional ex-vivo assay were correlated to the matched patient response in ~200 clinical scenarios. Training of an algorithm was developed from these ‘validated’ data, and the M-Score was derived, which predicts positive vs. negative response. D) To date, more than 400 patients have been evaluated by CA(N)script. (Note: here is a subset of clinical validation published in Nature Communications, 2018; Majumder et al. - Table 5.1) Table 1. CA(N)script Information for a subset of breast cancer patients. E) Predicted clinical response to conventional and immune checkpoint blockade.

Fig. 3. Model development to study NK cell spatial heterogeneity under pressure of therapy. A) CA(N)script was performed as described in Figure 2 using triplicate slices. Following 72h treatment with various conventional and immuno-mediatory therapies, listed in Table S1 above, tissue was examined for expression and localization of CD56+ (indication of NK cells). Based on regional (tumor and normal stroma) heterogeneity determined by clinical pathology, biomarkers were quantified between various control and therapy treated cohorts. B) Pathway analysis indicates variability in CD56- spatial (regional) heterogeneity in unsupervised (vehicle) and under drug pressure. Note: Green bars overlapped to demonstrate distribution in cases of positive M-Score. C) Quantification of CD56+ tumor-stromal determined by differential expression between vehicle and drug treatment (N=15).

Fig. 4. Alterations to NK cell spatial heterogeneity under drug pressure, ex-vivo, associates to predicted clinical response. Tissue from HER2/ERBB2 breast cancer patients (n=18) were interrogated ex-vivo by conventional chemotherapies and immune checkpoint blockade. Data expressed as differentially expressed cytokines (listed above graph), averaged among all patients.

Fig. 5. Tumor microenvironment modulated by pro- and anti-inflammatory cytokines, which associates to predicted response, ex-vivo. Tumor tissue from HER2/ERBB2 breast cancer patients (n=18) were interrogated ex-vivo by conventional chemotherapies and immune checkpoint blockade. Data expressed as differentially expressed cytokines (listed above graph), averaged among all patients.

Fig. 6 Multiplexed IHC captures spatial heterogeneity of tumor infiltrating lymphocytes (TILs) in CANscript™ Formalin-fixed paraffin-embedded CANscript breast tumor tissues (CD8 and Perforin-treated at T₁, stained for Pan-cytokeratin (magenta), CD3 (green), CD4 (yellow), CD8 (white), shown with DAPI (blue) and overlay of all markers). Image depicting IFNγ or IFNα, which enables downstream quantitative analysis of spatial heterogeneity.

CONCLUSIONS: Predicting clinical response is a holy grail of breast cancer treatment. Here, we determined that immune cells, such as NK, are dynamic in response to conventional and immunotherapeutic modalities, which contribute to therapy response or resistance. Using CA(N)script, a completely patient-autologous tumor model, we are able to study the dynamics of within the microenvironment as well as tumor-reflex to drugs, which aids in the prediction of treatment—a paradigm shift in personalized medicine.