Conclusions Genetic deletion or pharmacological inhibition of NOX2 sensitized AML cells to daunorubicin induced killing in hypoxic environments. NOX2 may thus be a target for overcoming chemoresistance in AML cells in the hypoxic bone marrow environment.

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Background Pancreatic ductal adenocarcinoma (PDAC) is a highly aggressive malignancy marked by poor prognosis and profound drug resistance characterized in more than 90% of cases by KRAS mutations. To recapitulate central aspects of PDAC, we employed genetically engineered mouse models presenting KrasG12D pancreas specific expression. Through a high-throughput combination drug screen with trametinib as backbone we identified a high synergism with the multikinase inhibitor nintedanib, preferentially in mesenchymal PDAC, a subtype of this disease characterized by poor prognosis and therapeutic resistance. This combinatorial treatment, that led to the induction of apoptosis in vitro and disease regression in vivo, was accompanied by a strong tumor infiltration of CD8 positive T cells.

Materials and Methods To characterize the treatment-induced adaptive immune cell infiltration in vivo, we performed orthotopic transplantations of KRAS-driven murine PDAC cell lines presenting mesenchymal and epithelial morphology. The derived control and nintedanib + trametinib treated PDAC tumors were analyzed by multi-color immunofluorescence stainings. We compared the findings to high parameter flow cytometry results.

Results Confocal microscopy of the immunofluorescence stainings revealed an overall increase of tumor-infiltrating lymphocytes (TIL) in the tumors upon combinatorial treatment with substantial differences in quantity and spatial distribution. Tumors derived from a PDAC cell line of epithelial morphology were characterized by few TIL mainly located at the invasive margins of the tumors, while tumors derived from a mesenchymal PDAC cell line showed a strong increase of TIL even in the center of the tumor mass. Furthermore, an increased ratio of CD8 positive cytotoxic T cells to CD4 positive helper T cells as well as a decrease of Foxp3 and CD4 positive regulatory T cells could be observed for tumors derived from the mesenchymal PDAC cell line under combinatorial treatment. To investigate if the observed recruitment of T cells was indispensable for treatment efficacy of the combinatorial therapy, we orthotopically transplanted the mesenchymal PDAC cell line in immunodeficient CD3-Knockout (CD3ko) mice and applied an analogous combinatorial treatment scheme. In the CD3ko mice, the combinatorial treatment did not lead to an increased survival or tumor regression as observed in immunocompetent mice. However, flow cytometry and immunofluorescence stainings revealed an increase of B cells upon nintedanib + trametinib treatment.

Conclusions Our findings indicate a reduced efficacy of the combinatorial treatment in T cell deficient mice, underlining the importance of T cells in treatment-induced anti-tumor responses and enlarging the understanding of the role of TIL in PDAC.