CALRETICULIN EXPOSURE ON MALIGNANT BLASTS NEUTRALIZING EXTRACELLULAR CHP-1 IMPAIRS TUMOR

The presence of calreticulin on the surface of malignant blasts is associated with enhanced NK cell cytotoxicity and secretion events, both in AML patients and in vivo in mice. The ability of calreticulin to stimulate NK cells relies on CD11c+CD14high cells that, upon exposure to CRT, express higher levels of IL-15Rα, maturation markers (CD86 and HLA-DR) and CCR7. CRT exposure on malignant blasts also correlates with the upregulation of genes coding for type I interferon. This suggests that CD11c+CD14high cells have increased capacity to migrate to secondary lymphoid organs, where they can efficiently deliver stimulatory signals (IL-15Rα/IL-15) to NK cells. These findings delineate a multifaceted, clinically relevant mechanism whereby surface-exposed calreticulin favors NK-cell activation in AML patients.

Conclusions
Combined Treg depletion/whole tumor vaccination therapy is effective in a poorly infiltrated B16 melanoma model. Combined treatment promotes T cell infiltration and activation. In mice achieving a partial response, treatment effects on the immune landscape were observed to decay over time suggesting a return to immune equilibrium. Further studies to explore the mechanistic basis of this observation are underway.

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P03.25 NEUTRALIZING EXTRACELLULAR CHP-1 IMPAIRS TUMOR GROWTH AND METASTASIS FORMATION

Background
Found in the extracellular compartment, Heat Shock Proteins (HSPs) are actively secreted proteins that modulate the tumor behavior. Extracellular HSPs play a unique role as extracellular chaperones and receptors-binding molecules, favoring the establishment and maintenance of different cancer hallmarks, including immune modulation and evasion. CHP-1, is a ubiquitously expressed protein with chaperone activity and its high expression correlates with high tumor grade and lymph node positivity in different breast and lung cancer subtypes. In addition, CHP-1 is actively and uncharacteristically secreted by cancer cells in the tumor microenvironment (TME).

Materials and Methods
Sera cancer patients were analyzed for the presence of CHP-1. To assess the role of extracellular CHP-1 (eCHP-1) in the TME, in vitro experiments on different cell populations have been performed. To dissect the molecular mechanisms, through which eCHP-1 induces cancer progression, have been analyzed specific signaling pathways in cancer and immune cells. Immune cell composition in presence of eCHP-1 in tumors has been identified using flow-cytometry. The characterization of CHP-1 inhibition as therapeutic approach has been conducted in breast and colon cancer pre-clinical models.

Results
eCHP-1 activates an autocrine signaling through TLR2, TLR4 and LRP1, promoting tumor progression and metastasis formation in different pre-clinical models. Moreover, eCHP-1 can modulate the immune composition of the TME, making interesting the analysis of its inhibition in cancer immunotherapy.
Abstracts

Conclusions eCHP-1 represents a easy accessible protein for diagnosis and targeting in very aggressive cancers.

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P03.27 Role of NOX2 for hypoxia-induced chemoresistance in acute myeloid leukemia

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Background Relapse of acute myeloid leukaemia (AML) may arise from residual chemoresistant leukemic cells. A hypoxic tumor microenvironment, such as the bone marrow, is known to enhance chemoresistance in various forms of cancer, including AML. Hypoxia inducible factor 1 alpha (HIF-1α) is an important mediator of cellular adaptation to hypoxia. HIF-1α is a constitutively expressed transcription factor that is rapidly degraded under normoxic conditions after hydroxylation by oxygen sensors, such as the HIF prolyl hydroxylases (PHDs). However, under hypoxic conditions the oxygen sensors lose the ability to induce the degradation of HIF-1α resulting in its stabilization and translocation to the nucleus where it induces the transcription of genes associated with glucose metabolism, angiogenesis, and cell survival. This may result in proliferation of malignant cells, impaired tumor cell differentiation and chemoresistance. Reactive oxygen species (ROS) have been shown to inhibit PHDs and may thereby stabilize HIF-1α, and may thus contribute to chemoresistance. AML cells may generate ROS via the myeloid NADPH oxidase NOX2. We therefore hypothesized that NOX inhibitors would decrease chemoresistance in a hypoxic environment.

Materials and Methods The wild type (WT) AML cell line PLB-985 and its NOX2 knocked out (KO) counterpart were cultivated for five days in hypoxia (1% oxygen) or normoxia (21% oxygen) in the presence or absence of the NOX inhibitors histamine dihydrochloride (HDC), diphenylethylenodionium (DPI) and GSK2795039. Thereafter cells were exposed to the chemotherapeutic agent daunorubicin for 48 hours (in hypoxia or normoxia) and cell death was determined using the XTT assay. Stabilization of HIF-1α was measured either by western blot or flow cytometry. Differentiation of cells was quantified by measuring the expression of CD14 and CD11b by flow cytometry.

Results Hypoxia reduced the sensitivity of WT PLB-985 cells to daunorubicin induced cell death (P < 0.05, n=4) whereas NOX2 KO cells were equally sensitive to daunorubicin in hypoxia and normoxia (P > 0.5, n=4). Furthermore, NOX2 KO AML cells displayed increased sensitivity to daunorubicin induced killing compared with PLB WT cells in a hypoxic environment (P < 0.05, n=4). Preliminary results show that pharmacological NOX inhibition using DPI enhanced the sensitivity of WT AML cells to daunorubicin induced killing. These results suggest that functional NOX2 contributes to chemoresistance in a hypoxic environment. As expected, hypoxia stabilized the expression of HIF-1α in AML cells. Preliminary results suggest that HIF-1α expression was reduced in the presence of NOX inhibitors.