was chosen, as significantly better uptake of the CPP was observed in AML cell lines and primary blasts compared to healthy PBMCs.²

Results Upon treating AML cell lines with the VLPs, we observed different SAMHD1-degradation capacities of the different Vpx homologs. SIVmac239 Vpx and HIV-2 7312a Vpx were most efficiently loaded into the VLPs, showed the highest SAMHD1-degradation and improved ara-C sensitivity up to 80-fold. In contrast, HIV-2 Rod9 Vpx did not show any SAMHD1 degradation or improvement in ara-C sensitivity despite its high packaging efficiency in the VLPs. As for the CPPs, CPP44 bound to 67aaVpx showed better uptake and SAMHD1 degradation compared to the TAT bound 67aaVpx in THP-1 cells, which is an AML cell line with high SAMHD1 expression levels. Upon co-treatment with ara-C, up to a 5-fold reduction in IC50 was observed when treated with CPP44-bound 67aaVpx. In order to increase the efficiency further, full-length Vpx-bound CPPs will be prepared, and trials using these CPPs are currently underway.

Conclusions

We demonstrate that inducing SAMHD1 degradation by Vpx delivered via VLPs or CPPs efficiently improved ara-C sensitivity in AML cell lines. Combining a Vpx delivery system with treatments containing ara-C might improve treatment outcomes in SAMHD1-high patients who are otherwise non-responsive.

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Disclosure Information R. Nair: None. H. Baldauf: None.

P01.05

DECIPHERING THE FUNCTION OF THE UBIQUITIN-PROTEASOME-SYSTEM IN REGULATING THE IMMUNE CHECKPOINT PROTEIN B7-H3 (CD276) IN NON-SMALL CELL LUNG CANCER

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10.1136/jitc-2021-ITOC8.9

Background Cancer cells use the expression of immune checkpoint proteins on their surface to evade immune responses. Targeting these checkpoints with antibodies has substantially advanced cancer therapy in the past years, especially the treatment of lung cancer. However, the prognosis of metastatic lung cancer patients still remains poor and lung cancer remains to be the leading cause of cancer death worldwide. Further therapeutic concepts are therefore urgently needed.

It has been shown that protein expression levels of the immune checkpoint protein PD-L1, a member of the B7 protein family, is regulated by the ubiquitin-proteasome system (UPS). Ubiquitin-ligases (E3-ligases) and deubiquitinating enzymes that regulate immune checkpoint levels on the cell surface are therefore considered promising potential drug targets. Inhibiting enzymes that increase immune checkpoint surface levels might increase the anti-cancer immune response.

Here, we investigate whether another B7 family member, immune checkpoint protein B7-H3, is regulated by the UPS in non-small cell lung cancer (NSCLC).

Materials and Methods B7-H3 expression in NSCLC cell lines and patient samples was evaluated using mRNASeq data from open databases. Immunoblotting and FACS were used to analyse total endogenous protein levels and surface expression of B7-H3 in different NSCLC lines under normal growth conditions and in response to various inhibitors (MG-132, Chloroquine (CQ) and Cycloheximide (CHX)). Immunoprecipitation of FLAG-tagged B7-H3 followed by a TUBE IP using ubiquitin-binding beads and in-vivo ubiquitylation assays based on co-overexpression of HA-tagged ubiquitin and/or HA-tagged K48/K63-linkages specific ubiquitin together with FLAG-tagged B7-H3 or FLAG-tagged B7-H3 K526R mutant in HEK-93T cells were performed to analyse ubiquitination on B7-H3. Mass spectrometry analysis of FLAG-purified B7-H3 was performed to identify possible interaction partners.

Results Database analysis revealed that B7-H3 expression is higher in lung cancer samples than in healthy lung tissue. We found that B7-H3 is highly expressed in different NSCLC lines on RNA and protein levels. Treatments with either proteasomal (MG-132) or lysosomal (CQ) degradation inhibitors alone showed only minor effects on B7-H3 protein abundance. However, CHX treatment of H1437 cells decreased B7-H3 over time and this decrease was recovered by adding MG-132 or CQ suggesting that both the lysosome as well as proteasome are involved in the degradation of B7-H3. In vivo ubiquitination and TUBE assay showed K48 and K63 B7-H3 ubiquitination. Mass spectrometry analysis of FLAG-tagged purified B7-H3 revealed E3-ligase Trim21, which has recently been identified as a ligase of PD-L1 in lung cancer lines, as a potential interaction partner. Further experiments are planned to validate the result and to identify other UPS-related enzymes involved in post-translational B7-H3 surface level regulation.

Conclusions Our experiments indicate that immune checkpoint B7-H3 levels are regulated by the ubiquitin-proteasome system in NSCLC lines. With further experiments, we aim to identify UPS-related enzymes that stabilize B7-H3 on the cell surface. Pharmacological inhibition of such enzymes might reduce the immune checkpoint's surface levels and increase anti-tumour immune responses.

Disclosure Information M. Kurz: None. L. Rieger: None. P. Giansanti: None. B. Kuster: None. F. Bassermann: None.

P01.06

OVERWEIGHT AND OBESITY AS BIOMARKERS FOR SURVIVAL OUTCOMES AND IMMUNE RELATED ADVERSE EVENTS UNDERGOING IMMUNOTHERAPY – A SYSTEMATIC REVIEW AND META-ANALYSIS

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10.1136/jitc-2021-ITOC8.10

Background The impact of overweight/obesity in cancer patients treated with immune checkpoint inhibitors (ICIs) is controversial. To further contribute to this debate, we

performed a systematic review and meta-analysis of published articles evaluating the effects of overweight/obesity on survival and immune-related adverse events (irAEs).

Materials and Methods In analogy to Cochrane recommendations, systematic literature searches included all published articles in PubMed until February 2021 with key terms ‘obesity’ and ‘overweight’ and ICI treatment irrespective of cancer entity and ICI used. Further selection criteria for meta-analysis included WHO cut-offs for overweight/obesity. For the random effects meta-analysis, we used Hazard Ratios (HR) for overall and progression-free survival (OS, PFS) and Odds Ratios (OR) for occurrence of irAEs with corresponding 95% confidence intervals (95%CI), respectively.

Results A total of 30 studies (12,895 patients, 38% female) selected for meta-analysis revealed a superior survival of overweight/obese patients (PFS: HR 0.9, 95%CI 0.77-1.04, $p = 0.11$; OS: 0.74, 95%CI 0.63-0.92, $p = 0.0005$) compared to normal weight patients. In subgroup analyses based on sex, overweight/obese male patients showed increased survival (PFS: HR 0.79, 95%CI 0.63-1.00, $p = 0.05$; OS: 0.71, 95%CI 0.58-0.86, $p = 0.0005$), whereas overweight/obese female patients had similar survival probabilities compared to their normal weight counterparts (PFS: HR 1.01, 95%CI 0.69-1.47, $p = 0.96$; OS: HR 0.73, 95%CI 0.48-1.10, $p = 0.13$). Underweight patients showed inferior survival (PFS: HR 1.48, 95%CI 1.07-2.04, $p = 0.02$; OS: HR 1.86, 95%CI 1.13-3.04, $p = 0.01$). In addition, overweight/obese patients had a higher risk of developing irAEs with grade ≥ 3 (OR 1.91, 95%CI 1.18-3.10, $p = 0.008$).

Conclusions Our meta-analysis revealed that overweight/obesity is a beneficial factor for PFS and OS in a mixed cohort of cancer patients undergoing ICI treatment accompanied by an increased risk of severe irAEs. The differences between overweight/obese males and overweight/obese females might point to sex specific adipose distribution patterns and interactions of sex steroids on a molecular level. A significant number of studies included underweight patients into normal weight control groups which led to a compromised interpretation of the data and should be addressed in future studies.

Disclosure Information P. Trinkner: None. S. Günther: None. M. von Bergwelt: None. D. Cordas dos Santos: None. S. Theurich: None.

P01.07

DEPLETION OF CD206^{HIGH} TUMOUR-ASSOCIATED MACROPHAGES USING A NANOCONJUGATE LIMITS TUMOUR BURDEN & DISSEMINATION IN METASTATIC TRIPLE NEGATIVE BREAST CANCER IN MICE

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10.1136/jitc-2021-ITOC8.11

Background Anti-inflammatory (M2) tumour-associated macrophages (TAMs) exert protumoural roles through angiogenesis, immunosuppression and resistance to therapies.¹ M2 TAMs express the mannose receptor, CD206,² excellent marker for targeted therapies. We have previously identified a peptide called mUNO² that specifically binds to CD206 on M2 TAMs. Aiming to dissect the role of CD206^{high} M2 TAMs in the tumour progression and immunosuppression, we depleted them using an mUNO and doxorubicin (Adriamycin®)-containing polymer-drug nanoconjugate (St-PGA-DOX-mUNO, ‘Oxi-mUNO’) where the polymer backbone is branched polyglutamic acid (St-PGA).³

Materials and Methods We compared OximUNO with free DOX and the untargeted nanoconjugate St-PGA-DOX. To study the in vitro cytotoxicity of the nanoconjugates, we used M2 and M1 skewed macrophages derived from human blood buffy coat. To study the in vivo homing of nanoconjugates we used an orthotopic triple negative breast cancer (TNBC, 4T1 cells) model and a TNBC experimental metastases model in immunocompetent mice. For in vivo therapeutic efficacy studies, we used orthotopic and experimental metastases models of TNBC, and administered the compounds intraperitoneally (i. p.).

Results In vitro, OximUNO showed 39% higher toxicity to the primary human M2 macrophages than St-PGA-DOX, and 31% lower toxicity to the M1 macrophages than St-PGA-DOX. In vivo, OximUNO showed no change in creatinine or alanine aminotransferase values, indicating no toxic effects to the kidneys or liver. Compared to control St-PGA, i.p.-administered St-PGA-mUNO, showed improved homing to M2 TAMs in both orthotopic and experimental metastases models with low accumulation in the liver. In the orthotopic treatment study, only OximUNO significantly reduced the tumour volume and showed 56% and 38% less lung metastases than DOX and St-PGA-DOX, respectively. Additionally, DOX and St-PGA-DOX produced a significant bodyweight loss whereas OximUNO did not. Importantly, OximUNO treatment resulted in 2-5-fold increase in the ratio of CD8⁺/FOXP3⁺ expression, suggesting a shift in the immune landscape towards an immunostimulatory profile. In the experimental metastases model, OximUNO monotherapy resulted in the highest reduction of lung metastases, and this effect correlated with a significant reduction in CD206^{high} M2 TAMs; whereas no significant effect on M2 TAMs population was observed with DOX or untargeted nanoconjugate.

Conclusions Our data suggests that the elimination of CD206^{high} M2 TAMs with OximUNO suppresses spontaneous and experimental metastases in safe manner, shifts immune landscape towards immunostimulatory and could therefore be a potential treatment option for TNBC patients.

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