Conclusion Here we have shown for the first time that FTY720 can sensitise ER negative breast cancer to docetaxel. We further demonstrate that encapsulation of free drugs in nanoparticles can improve targeting, provide low off-target toxicity and enhance antitumour efficacy offering potential therapeutic use of FTY720 in clinical breast cancer treatment.

PO-433 THE MODULATION OF REDOX HOMEOSTASIS AND INDUCTION OF FERROPTOTIC CELL DEATH IN HEPATOCELLULAR CARCINOMA AS AN ANTICANCER STRATEGY

[1,2,3,4]J Liese*, [4]J Lippmann, [2,4,5]S Fulda. [1]*University Hospital of Giessen, Department of General- Visceral- Thoracic- Transplant and Pediatric Surgery, Giessen, Germany*; [2]*German Cancer Consortium DKTK, German Cancer Consortium DKTK, Heidelberg, Germany*; [3]*German Cancer Consortium DKTK, German Cancer Research Center DKFZ, Heidelberg, Germany*; [4]*Goethe-University, Institute for Experimental Cancer Research in Pediatrics, Frankfurt, Germany*; [5]*German Cancer Research Center DKFZ, German Cancer Research Center DKFZ, Heidelberg, Germany*

10.1136/esmoopen-2018-EACR25.457

Introduction Ferroptosis has recently been identified as a form of programmed cell death caused by an accumulation of lipid reactive oxygen species (ROS). However, little is yet known about the role in hepatocellular carcinoma (HCC) and its signalling mechanism as well the modulation of ROS.

Material and methods Human HCC cell lines were treated with different concentrations of ROS modulators (Auranofin, Erastin, BSO). Cell death was determined by analysis of PI-stained nuclei using flow cytometry. ROS production and lipid peroxidation were analysed at early time points before cell death starts. For mechanistic studies we performed Western Blot and a Proteome array. Different inhibitors of cell death target proteins, ROS-scavengers as well as lipoxygenase inhibitors were used. To investigate the functional relevance of NAPDH oxidases (NOX) 1 and 4 for ROS modulation and ferroptosis we genetically silenced its genes using three distinct siRNAs and we used the NOX1/4-inhibitor GKT137831.

Results and discussions Compared to the single treatment, Auranofin/BSO-cotreatment as well as Erastin/BSO-cotreatment acted in concert to trigger cell death and to reduce cell viability of HCC cells in a dose- and time-dependent manner. Furthermore, both cotreatments induce ROS production, lipid peroxidation and ferroptotic cell death, which could be inhibited by the use of Ferrostatin-1 (inhibitor of lipid peroxidation) and Liproxstatin-1 (specific inhibitor of ferroptosis). The broad-range caspase inhibitor zVAD.fmk failed to rescue cells from Auranofin/BSO- or Erastin/BSO-cotreatment induced cell death. No activation of caspases-3 could be seen in the proteome profiler apoptosis assay. Importantly, the selective lipoxygenase (LOX) inhibitor Baicalain and the pan-LOX inhibitor NDGA protect HCC cells from Auranofin/BSO- and Erastin/BSO-cotreatment stimulated lipid peroxidation, ROS generation and cell death, indication that the induction of ferroptosis may bypass apoptosis resistance of HCC cells. Mechanistic studies showed that Auranofin/BSO-cotreatment decreased TrxR-activity, led to Nrf2 accumulation and promoted the activation of HO-1. In contrast, NOX 1 and 4 were involved in Erastin/BSO-mediated cell death and the use of the NOX1/4-inhibitor GKT137831 rescued HCC cells from the Erastin/BSO-induced cell death.

Conclusion By providing new insights into the molecular regulation of ROS and ferroptosis, our study contributes to the development of novel treatment strategies to reactivate programmed cell death in HCC cells.

Published online 29 June 2018

PO-434 AT1413 ANTIBODY DERIVED FROM A CURED AML PATIENT RECOGNISESA UNIQUE SIALYLATED CD43 EPITOPE SHARED BY AML, MDS AND MELANOMA CELLS

[1]G De Jong, [2]L Bartels, [2]M Kedde, [3]E Verdegaal, [2]E Yasuda, [2]P Van Helden*, [2]K Wagner, [2]R Schotte, [2]H Spits, [1]M Hazenberg. [1]*Academic Medical Center, Department of Hematology, Amsterdam, The Netherlands*; [2]*AIMM Therapeutics, AIMM Therapeutics, Amsterdam, The Netherlands*; [3]*LUMC, Department of Medical Oncology, Leiden, The Netherlands*

10.1136/esmoopen-2018-EACR25.458

Introduction AT1413 is an antibody derived from B cells of an AML patient who was cured following allogeneic hematopoietic stem cell transplantation. It recognises a sialylated epitope on CD43 (CD43s), which is expressed on myeloid cells but not on B and T cells and is over-expressed on acute myeloid leukaemia (AML) and myelodysplastic syndrome (MDS) cells. AT1413 kills AML cells *in vitro* and *in vivo* via antibody dependent cell-mediated cytotoxicity (ADCC) suggesting that AT1413 played a role in the graft versus leukaemia response of this patient. Because CD43 is broadly expressed in non-hematopoietic cells we explored whether CD43s is present on non-hematopoietic tumours.

Material and methods AT1413 binding on a panel of tumour cell lines was analysed by flow cytometry. AT1413 was assembled into a bispecific T-cell engaging format (AT1413 bTCE) by linking the full-length AT1413 IgG to two single chain variable fragments against CD3e with a combination of site-specific enzymatic and chemical coupling. Two point mutations in the IgG heavy chain were introduced to prevent interactions between AT1413 bTCE and Fc gamma receptors. The cytotoxicity-inducing activities of naked AT1413 and its bTCE format were tested with PBMCs as effector and tumour cells as target cells using standard cytotoxic assays.

Results and discussions AT1413 bound to melanoma cell lines but not to pancreas carcinoma, colon carcinoma, or liver carcinoma. Expression on melanoma cells was confirmed by immunoprecipitation and western blot using a mouse anti-human CD43 antibody. AT1413 bound to 14 out of 21 patient-derived primary melanoma samples with varying intensities. AT1413 induced ADCC of melanoma cell lines and patient-derived melanoma cells. The level of ADCC correlated with CD43s expression levels. To increase the cytotoxicity inducing potential of AT1413 we generated a bTCE format and demonstrated that it induced strong cytotoxic T cell activities against melanoma cells *in vitro*. The efficacy of AT1413 and AT1413-bTCE on human melanoma cells *in vivo* in a xenograft mouse model is currently tested.

Conclusion The AT1413 antibody recognises a sialylated epitope on CD43 shared by melanoma, AML and MDS cells. Both the non-modified IgG- and the bispecific TCE form of AT1413 induce strong anti-tumour cytotoxic activities *in vitro* and *in vivo*. Because of its broad tumour reactivity and functional activities AT1413 has promising therapeutic potential.