PO-435 PHOTOACTIVATION OF NANOPARTICLES DELIVERED BY MESENCHYMAL STEM CELLS INDUCES OSTEOSARCOMA CELL DEATH IN *IN VITRO* 3D CO-CULTURE MODELS

[1]S Lenna*, [1]C Bellotti, [2]S Duchi, [2]M Ballestri, [2]E Martella, [3]B Dozza, [4]M Columbaro, [2]A Guerrini, [2]G Varchi, [1]DM Donati. [1]*Rizzoli Orthopaedic Institute, Osteoarticolar Regeneration Laboratory, Bologna, Italy*; [2]*Italian National Research Council, Institute of Organic Synthesis and Photoreactivity, Bologna, Italy*; [3]*Alma Mater Studiorum University of Bologna, Department of Biomedical and Neuromotor Sciences, Bologna, Italy*; [4]*Rizzoli Orthopaedic Institute, Laboratory of Musculoskeletal Cell Biology, Bologna, Italy*

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Introduction Osteosarcoma (OS) is a rare and aggressive tumour that mainly affects long bones of adolescents. Currently, OS patients are treated with a combination of multi-agent chemotherapy and surgery. However, 30% of patients do not respond to standard treatment. Therefore, innovative therapeutic agents are needed. Mesenchymal stem cells (MSCs) display a specific tumour-tropism and have been previously used in successful preclinical studies to deliver several therapeutic agents. Furthermore, the safety of genetically engineered MSCs was demonstrasted in ongoing clinical trial. The goal of the present study was to test *in vitro* whether MSCs could uptake photoactivable nanoparticles (NPs) and induce cell death of OS cells upon photoactivation.

Material and methods Ptl@PMMA NPs were produced by adding tetrasulfonate aluminium phthalocyanine (Ptl) to an aqueous solution of positively charged poly-methylmethacrylate (PMMA) nanoparticles. The photosensitizer Ptl is activated in near-infrared light allowing a deep tissue penetration. Human MSC lines, isolated from the bone marrow of multiple donors, were loaded with Ptl@PMMA NPs. The MSCs' ability to internalise and retain NPs, along with their migratory properties, were tested. Cell death upon photoactivation (PDT) was evaluated *in vitro*, on a monolayer co-culture of MSCs and OS cells and in 3D multicellular spheroids, generated via cell suspension in ultralow attachment plates

Results and discussions MSCs showed an internalisation rate of Plt@PMMA>95%, which did not alter cell viability and migratory capacity. When Ptl@PMMA-MSCs were co-cultured with a human OS cell line (SaOS-2) in monolayers, they efficiently triggered cell death upon PDT. In particular, AnnexinV/PI and CalceinAM/EthD staining showed 70% of cell death in the co-culture system. These results were also validated by a metabolic assay. Interestingly, in a 3D co-culture of the OS cell line MG63 and Ptl@PMMA-MSCs, we observed a marked reduction of the viability (<5%) measured by the ATP content 24 hours after PDT. A massive cell necrosis induced by the photoactivation of the Ptl in the whole spheroid mass was confirmed by CalceinAM/EthD staining and TEM imaging.

Conclusion For the first time, we demonstrated that photoactivation of MSCs loaded with Ptl@PMMA NPs can successfully induce OS cell death in a three-dimensional OS model. These results encourage further *in vivo* evaluation to demonstrate the specific targeting of Plt@PMMA loaded MSCs to the tumour stroma and the efficacy of PDT treatment

PO-436 RETARGETING T-CELL CYTOTOXICITY TO A UNIQUE SIALYLATED EPITOPE ON CD43 EXPRESSED BY ACUTE MYELOID LEUKAEMIA

[1]L Bartels*, [1]G De Jong, [2]E Yasuda, [2]V Kattler, [2]AQ Bakker, [2]J Villaudy, [2]PM Van Helden, [3]MD Hazenberg, [1]H Spits, [2]K Wagner. [1]*AIMM Therapeutics and Academisch Medisch Centrum, Experimental Immunology, Amsterdam, The Netherlands*; [2]*AIMM Therapeutics, AIMM Therapeutics, Amsterdam, The Netherlands*; [3]*Academisch Medisch Centrum, Hematology and Experimental Immunology, Amsterdam, The Netherlands*

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Introduction From the B cell repertoire of an acute myeloid leukaemia (AML) patient in long-term remission after hematopoietic stem cell transplantation we recently identified the antibody AT1413. AT1413 binds CD43s, a unique sialylated epitope on CD43 present on AML and myeloid cells but not on B and T cells. Besides its therapeutic potential as a naked antibody, AT1413 provides an interesting candidate for a bispecific T-cell engaging antibody (bTCE). bTCEs have been clinically validated as a powerful tool for harnessing the cytotoxicity of polyclonal T cells against a hematologic cancer. Simultaneous binding to a cancer surface antigen and the T-cell surface protein CD3ϵ mediates cancer cell recognition and T-cell mediated killing independent of the T-cell receptor specificity.

Material and methods To generate an AT1413 bTCE, we first modified AT1413 to abolish Fc-receptor interaction. Second, we assembled the bispecific by chemo-enzymatic linkage using a combination of a sortase-catalysed transpeptidation reaction and a subsequent cycloaddition reaction.

AML target cell lysis by T-cells was assessed *in vitro* in a cytotoxicity assay. Up-regulation of T-cell activation markers CD69 and CD25 and cytokine production were monitored as indicators for T-cell activation. T-cell proliferation was assessed. I*n vivo*, AT1413 bTCE was tested in two mouse models, one where human PBMCs were co-injected at the start of bTCE treatment and the other in which a human immune system (HIS) was engrafted at birth.

Results and discussions AT1413 bTCE was confirmed to retain dual binding capacity for both AML cells and CD3ϵ-expressing Jurkat cells. *In vitro*, AT1413 bTCE successfully induced T-cell mediated cytotoxicity against different CD43s expressing AML cell lines as well as primary AML blasts. Endothelial cells that have a detectable but considerably lower binding capacity for AT1413 remained unaffected. Besides cytotoxicity, T-cell activation and T-cell proliferation were observed and were dependent on the presence of target-expressing AML cells. I*n vivo* testing of AT1413 bTCE dosed at 2 mg/kg revealed potent AML tumour growth inhibition of 89%–99% in two mouse models when compared with a control bTCE. In the HIS model, normal human hematopoietic cells remained present in mice treated with AT1413 bTCE.

Conclusion Our results indicate that CD43s is a potential new target for T-cell engaging antibodies. Consequently, AT1413 holds therapeutic potential not only as an unmodified antibody, but also in a bispecific, T-cell engaging format.

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