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ORAL ABSTRACTS

703.CELLULAR IMMUNOTHERAPIES: BASIC AND TRANSLATIONAL

Bone Marrow Stroma Impairs CAR-T Cell Proliferation and Function: Mechanistic Insights

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Chimeric Antigen Receptor (CAR)-T cells have proven highly successful in the treatment of some haemopoietic neoplasms. However, in several cases the treatment fails and disease does not respond and/or relapses. Amongst the different factors impacting on a negative clinical outcome, an unfavourable immunosuppressive microenvironment has been proposed as playing a critical role, particularly in the context of acute myeloid leukemia. We hypothesised that bone marrow mesenchymal stromal cells (BM-MSC) acquire immunosuppressive properties after exposure to the by-stander effects of activated CAR-T cells against leukemic cells. We used a CAR-T cell consisting of a CD33 binder and CD28z-OX40 costimulatory moiety. BM-MSC were exposed to the CAR following in vitro activation on CD33-coated plates for 24 hours. To model the different ways by which stromal cells can license their immunosuppressive activity, BM-MSC were exposed to the supernatant harvested from the cultures of activated CARs or in direct contact with the activated CARs themselves. In the first case, after incubation with the CAR-conditioned medium (CAR-cm), BM-MSCs were investigated for their transcriptome by RNASeq. Gene ontology analysis of the transcriptome revealed a strong NF-κB and STAT1 signature in BM-MSC exposed to the CAR-cm compared to those which were not exposed. Amongst the most upregulated molecules, we identified several chemokines (CXCL10, CCL8, CXCL8, and CXCL9), PDL1 and CECAM1, IL-6, indoleamine-2,3 dyoxigenase (IDO), and most prominently PTGES and PTGS2. We then interrogated the immune functional profile by incubating by CAR-cm licensed BM-MSC with fresh CD33-activated CAR-T cells. We observed that CAR proliferation, cytotoxicity and cytokine production was significantly inhibited. CAR function was restored if Rel-A - a REL-associated protein critically involved in NF-κB heterodimer formation, nuclear translocation and activation - was inhibited in BM-MSC by shRNA before exposure to CAR-cm. We previously described that BM-MSC undergo apoptosis when in contact with activated cytotoxic T cells and that this also triggers their immunosuppressive capability. Therefore, we evaluated the impact of cell contact dependent mechanisms on BM-MSC licensing. CD33-activated CAR-T cells were incubated with BM-MSC and the proportion of annexin-V positive BM-MSC were assessed after 4 hours. We observed that a large fraction of BM-MSC underwent apoptosis and that this was dependent on caspase activation, but not RIP kinase 3, and the release of cytolytic granules in a contact-dependent fashion. The supernatant of apoptotic BM-MSC but not the terminally apoptotic and neither the live cells exhibited a strong inhibitory activity on CAR-T cell proliferation, cytotoxicity and effector cytokine production. Similarly to what described with the CAR-cm induced profile, BM-MSC immunosuppression required NF-κB and was effected via the COX2 pathway. We finally tested the impact of apoMSC in vivo in a xenograft model of AML (Fig. 1). CD33-positive KG1 cells were injected in NSG mice and 2 weeks later, CD33-specific CAR-T were administered with or without (controls) apoMSC. Peripheral blood samples were collected 7, 14, and 21 days after. We observed an inhibitory effect of apoMSc on the in vivo expansion of CAR-T cells which was accompanied by disease progression. Accordingly, the survival of the mice treated with apoMSC was markedly reduced compared to those receiving CAR-T cells only.

We conclude that BM-MSC can be induced to acquire immunosuppressive properties by activated CAR-T cells through 2 different but overlapping mechanisms, both dependent on NF-κB and COX2 signalling pathway (Fig. 2). Our work provides critical information to overcome resistance to CAR-T cell treatment by neutralising the negative effects of the stromal microenvironment.

Disclosures Dazzi: *AstraZeneca:* Current Employment. **Tettamanti:** *CoImmune Inc:* Research Funding. **Kozlowska:** *AstraZeneca:* Current Employment.

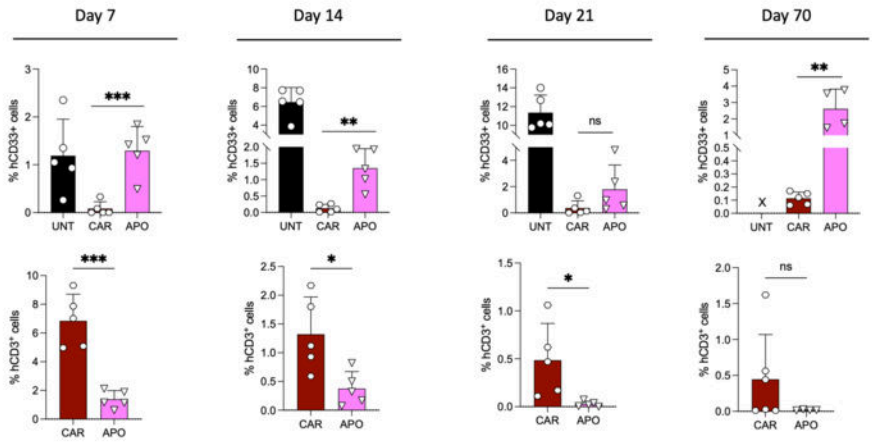

Figure 1. Apoptotic BM-MSCs impairs in vivo CAR-T cell expansion and favours disease progression. A leukaemia xenograft model was established by injecting CD33⁺ KG-1 AML cells into NSG mice (5x10⁶/mouse). Fourteen days later, mice were treated with CD33.CAR-T (10x10⁶/mouse) alone or together with apoptotic BM-MSCs (2x10⁶/mouse). Peripheral blood was sampled at day 7, 14, 21 and 70 after treatment. Percentage of CD33⁺ AML cells (A) and CD3⁺ CAR-T cells (B) were measured via flow cytometry. Results are represented as mean ± SD (n=5 mice/group). * P < 0.05, ** P < 0.01, *** P< 0.001

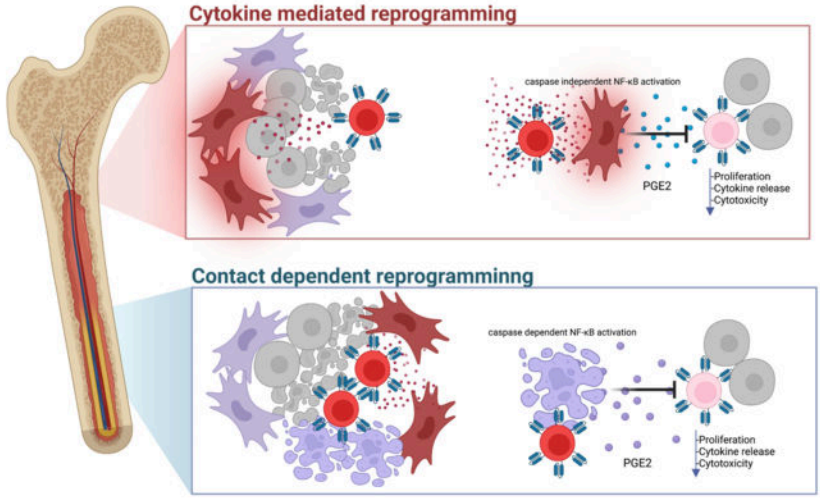

Figure 2. Activated CAR-T cells directly imparts bone marrow stroma to acquire immunosuppressive properties by two modalities. In this study we have shown that activated CAR-T cells promote BM-MSC to acquire an immunosuppressive profile. This can be induced either by the secretome induced by binding with the antigen or after cell contact whereby BM-MSC undergo caspase-dependent apoptosis. The mechanisms responsible for immunosuppression are largely overlapping. In both cases, the induction is NF-κB dependent and effected through the COX2 pathway.

Figure 1

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