by ELISA. Inhibitors of RANKL were then tested in vitro. We selected several RANKL inhibitors: anti-RANKL antibody, RANK-Fc, Isoliquiritigenin and Gallocatechin gallate. The efficacy of these inhibitors was indirectly evaluated with the murine macrophage RAW264.7 cell line which undergoes, in the presence of RANKL, an osteoclast differentiation (TRAP and Cathepsin K expression). The efficacy of RANKL inhibitors was then evaluated, in this model, by RT-qPCR. Apoptosis and proliferation of the cancer cell lines in the presence of the inhibitors were also analyzed.

**Results** RANKL/PD-L1 expression profile on specimens from each breast cancer subtype showed that both immunosuppressive molecules are expressed by all breast cancers with a significantly more intense immunoreactivity for triple negative breast cancers. Most of the cell lines expressed both proteins. We found that RANKL is secreted in their extracellular environment. RANKL inhibitors are efficient and will be tested in vivo.

**Conclusions** Several murine triple negative breast cancer cell lines will be sub-cutaneously injected in mice and the efficacy of both RANKL and PD-L1 inhibitors will be evaluated (separately or in combination). The infiltration of tumor microenvironment by different immune cell populations, the presence of metastasis and the tumor growth will be analyzed.


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**P09 Young Researchers Session**

**P09.01** ADOPTIVE CELL THERAPY OF HEMATOLOGICAL MALIGNANCIES USING CYTOKINE-INDUCED KILLER CELLS RETARGETED WITH MONOCLONAL ANTIBODIES

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Background Cytokine-Induced Killer (CIK) cells are a population of effector cells that represents a promising tool for adoptive cell therapy. They are easily expandable \textit{ex-vivo}, safe, and exert cytotoxicity against a broad range of tumor histotypes.\textsuperscript{1} We recently reported that they have a relevant expression of FcyRIIIa (CD16a), which can be exploited in combination with clinical-grade monoclonal antibodies (mAbs) to redirect their cytotoxicity in an antigen-specific manner, to improve their antitumor activity.\textsuperscript{2} Indeed, the engagement of CD16a on CIK cells leads to a potent antibody-dependent cell-mediated cytotoxicity (ADCC) against ovarian cancer both \textit{in vitro} and \textit{in vivo}. Based on this observation, we investigated whether CIK cells can be specifically retargeted against B-cell malignancies by combination with anti-CD20 mAbs, namely Rituximab\textsuperscript{a} (RTX) and Obinutuzumab\textsuperscript{b} (OBI).

Materials and Methods CIK cells were obtained from peripheral blood mononuclear cells of healthy donors, and stimulated \textit{in vitro} with IFN-\gamma, CD3 mAb and IL-2 for 14 days; fresh IL-2 was provided every 3–4 days. CIK cell phenotype was analyzed by multicolor flow cytometry; cytotoxic activity was assessed by calcein AM-release assay against B-cell lines, primary samples and patient-derived xenografts (PDX) obtained from B-cell lymphoma patients after written informed consent.

Results The combination with both RTX and OBI significantly increased specific CIK cells lysis against several CD20-expressing lymphoma B cell lines, primary tumors from B-cell lymphoma patients and an established PDX, compared to the combination with a control mAb (cetuximab, CTX). NK-depletion demonstrated that the mAb-mediated cytotoxicity is accountable to the CIK cells fraction within the bulk population since no difference in the lytic activity was detected in the absence of NK cells. In addition, these results are further supported by \textit{in vitro} preliminary experiments where the treatment with CIK cells in combination with OBI extensively reduced the growth of PDX and increased mice survival, compared to CIK cells or OBI administered alone.

Conclusions Here we proved that CIK cells can be retargeted with clinical-grade mAbs against CD20-expressing lymphomas. These data indicate that the combination of CIK cells with mAbs can represent a novel approach for the treatment of haematological malignancies.

REFERENCES


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