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 subtypes 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.

Disclosure Information C. Pilard: None. **P. Roncarati:** None. **E. Hendrick:** None. **A. Lebeau:** None. **D. Bruyère:** None. **T. Lerho:** None. **M. Ancion:** None. **C. Reynders:** None. **P. Delvenne:** None. **M. Herfs:** None. **P. Hubert:** None.

P08.04 NEOADJUVANT CHEMORADIOTHERAPY WITH SEQUENTIAL IPILIMUMAB AND NIVOLUMAB IN RECTAL CANCER (CHINOREC): A PROSPECTIVE RANDOMIZED, OPEN-LABEL, MULTICENTER, PHASE II CLINICAL TRIAL

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10.1136/jitc-2020-ITOC7.100

Background Immune checkpoint inhibitors (ICI), such as ipilimumab (anti-cytotoxic T-lymphocyte-associated protein 4; CTLA-4) or nivolumab (anti-programmed cell death protein 1; PD-1) have been proven to be an effective strategy in solid cancers. Nevertheless, ICI seem not to be effective in microsatellite stable (MSS) tumors, as those potentially lack an immunogenic priming. Radiotherapy is capable to induce an immunogenic cell death (ICD) and subsequently an immunogenic tumor immune microenvironment (TIME). Thus, the pro-inflammatory effect of radiotherapy might restore the susceptibility of MSS tumors to ICI, leading to more pronounced tumor shrinkage, as well as to an effective anti-tumor immune response.

Material and Methods In total 80 patients with a locally advanced rectal cancer (LARC) will be randomly assigned in a 30:50 ratio, to receive either standard of care (SOC) neoadjuvant chemoradiotherapy alone (CRT; 50 Gy in 2 Gy fractions with capecitabine 1650 mg/m^2/d over 25 working days) or concomitant with a single dose of ipilimumab 1 mg/kg on day 7 following sequentially 3 cycles of nivolumab 3 mg/kg Q2W starting on day 14. Patients will undergo surgery within 10 to 12 weeks post CRT. The primary endpoint is safety, tolerability and feasibility assessed by the latest Clavien-Dindo classification of surgical complications and the common terminology criteria of adverse events (CTCAE).

Results ClinicalTrials. gov identifier: NCT04124601. Serial liquid (plasma, serum, peripheral blood mononuclear cells) and tissue biopsies will be taken sequentially before, during and after neoadjuvant therapy. Secondary objectives are radiographic (mrTRG) and pathological (TRG) therapy response. Immune cell infiltrate of resected specimen, as well as genomic, transcriptomic, epigenomic and proteomic pattern of sequential liquid and tissue biopsies will be correlated with therapy response and clinical outcome.

Conclusion This is the first in human study, which uses neoadjuvant CRT in LARC patients with concomitant ipilimumab and nivolumab, applied in a sequential approach. A detailed understanding of therapy induced changes during neoadjuvant CRT with concomitant ICI in a human translation setting will allow the application of radiotherapy as a part of novel immunotherapeutic concept. This is an investigator-initiated trial (IIT), which received a research grant and the study medications from Bristol-Myers Squibb (BMS).

Disclosure Information J. Laengle: B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Significant; IIT, Research Grant & Study Medication from BMS. **I. Kuehrer:** None. **D. Pils:** None. **J. Kabiljo:** None. **K. Wöran:** None. **J. Stift:** None. **F. Herbst:** None. **B. Dauser:** None. **M. Monschein:** None. **P. Razek:** None. **S. Haegele:** None. **M. Herac:** None. **W. Hulla:** None. **C. Bitterman:** None. **F. Laengle:** None. **D. Tamandl:** None. **J. Widder:** None. **R. Schmid:** None. **M. Bergmann:** B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Significant; IIT, Research Grant & Study Medication from BMS.

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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10.1136/jitc-2020-ITOC7.101

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 *ex-vivo*, safe, and exert cytotoxicity against a broad range of tumor histotypes.[1] We recently reported that they have a relevant expression of FcγRIIIa (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.[2] Indeed, the engagement of CD16a on CIK cells leads to a potent antibody-dependent cell-mediated cytotoxicity (ADCC) against ovarian cancer both *in vitro* and *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® (RTX) and Obinutuzumab® (OBI).

Materials and Methods CIK cells were obtained from peripheral blood mononuclear cells of healthy donors, and stimulated *in vitro* with IFN-γ, 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 detectd in the absence of NK cells. In addition, these results are further supported by *in vivo* 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.

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The research leading to these results has received funding from Fondazione AIRC under IG 2018 - ID. 21354 project - P.I. Rosato Antonio

Disclosure Information A. Dalla Pietà: None. **E. Cappuzzello:** None. **P. Palmerini:** None. **R. Sommaggio:** None. **G. Astori:** None. **K. Chieregato:** None. **O. Perbellini:** None. **M. Tisi:** None. **C. Visco:** None. **M. Ruggeri:** None. **A. Rosato:** None.

P09.02 MAPPING AND TACKLING TUMOR AND CHEMOTHERAPY-INDUCED IMMUNE SUPPRESSION IN BREAST CANCER SENTINEL LYMPH NODES

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10.1136/jitc-2020-ITOC7.102

Background Breast cancer (BrC) is the most prevalent cancer in women worldwide. Unfortunately, still limited treatment options are available for the most aggressive subtypes (i.e. hormone receptor [HR] negative). The response to neoadjuvant chemotherapy (NACT) in patients with HR-negative BrC can in part be influenced by an effective anti-tumor immune response. The sentinel lymph node (SLN) is the first site where BrC-specific T cell priming will occur but unfortunately it is also a major target of BrC-induced immune suppression. Lymph-node resident dendritic cells (LNR-DC) were found to be suppressed in metastatic SLN.[1] In addition, this tumor-mediated immune suppression of LNR-DC is related to high-risk triple-negative BrC and may be a negative predictor for prognosis[1]. Preliminary data showed that NACT further reduced the activation status of LNR-DC. The goal of this study is to identify immune-enhancing agents that can counteract the tumor-mediated immune suppression of LNR-DC and promote tumor-specific T cell responses in order to improve therapy outcome in BrC patients upon NACT.

Materials and Methods Phenotypic analyses were performed on immune-cell subsets in human BrC SLN using multi-color flow cytometry. In addition, *ex-vivo* cultures with human BrC SLN-derived cells and *in vivo* mouse experiments were performed to study the therapeutic efficacy of Toll-like receptor (TLR)-ligands (R848 and CpG) and a STING-ligand (STING-L; 2'3'-c-di-AM(PS)$_2$(Rp,Rp)).

Results Higher rates of LNR-DCs, but with an apparently reduced activation state, were found in SLN of NACT-treated patients compared to patients treated with surgery only. A comparative *ex-vivo* study with SLN cultures on the effects of R848, CpG-B and STING-L showed R848 to be superior in terms of LNR-DC activation. In a *Krt14 (K14)-cre;Cdh1F/F;Trp53F/F* (KEP) BrC mouse model, the effects of intratumoral administration of TLR- and STING-L were determined in combination with doxorubicin. STING-L outperformed R848 and CpG-B in terms of controlling primary tumor growth. Of note, in human e*x-vivo* cultures CpG-B proved effective in LNR-DC activation when combined with a STAT3 inhibitor, leading to the boosting of mammaglobin-specific T cell responses, Th1 skewing, and a drop in CpG-induced Treg levels.

Conclusions In summary, intratumoral delivery of TLR- and STING-ligands in combination with NACT might be an interesting therapeutic approach in patients with high-risk HR-negative BrC, leading to SLN potentiation and enhanced antitumor T cell immunity. Future clinical studies should demonstrate the therapeutic benefit of this approach.

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