

Results: At odds with conventional PARP inhibitors, OX413 bound to and hyper-activated PARP1 with high affinity and in a dose-dependent manner, resulting in elevated cytotoxicity in multiple cancer (but not normal) cells irrespective of HR status. OX413 impaired DNA repair due to PARP trapping and rapid NAD⁺ consumption, leading to the accumulation of cytoplasmic chromatin fragments (CCFs). Consistent with this, OX413 triggered activation of the CGAS/STING pathway, CCL5 secretion and potentiation of tumor-targeting T cell responses. PARP1 engagement and STING activation were corroborated in vivo in EMT6 mouse mammary carcinomas, correlating with anticancer effects coupled to tumor infiltration by innate and adaptive immune cells. In MPA/DMBA-driven tumors, OX413 mediated considerable anticancer effects that could be exacerbated by PD1 inhibition.

Conclusions: Our results provide preclinical rationale for using OX413 to trigger metabolic exhaustion in cancer cells and initiate inflammatory responses that can be actioned by immune checkpoint inhibitors in patients bearing HRD as well as HRP tumors.

Legal entity responsible for the study: The authors.

Funding: Onxeo.

Disclosure: All authors have declared no conflicts of interest.

<https://doi.org/10.1016/j.annonc.2022.07.1769>

1692P ImmTAC redirect T cells against patient-derived tumour organoids and three-dimensional melanospheres: Effects augmented by type I interferons

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Background: ImmTAC molecules are TCR-anti-CD3 bispecific fusion proteins that can redirect polyclonal T cell activation against cancer cells. Tebentafusp, a gp100-directed ImmTAC, has demonstrated survival benefit in metastatic uveal melanoma¹. Here, patient-derived tumour organoids (TO)² and 3-dimensional multicellular melanospheres were evaluated as tumour models to study ImmTAC activity.

Methods: 3D melanospheres were generated by low adherence culture of melanoma cell lines (MEL202, MEL624, 92.1, MP-41, A375, IGR-1, Mewo) and their melanin synthesis genes were quantified by qPCR. The capacity of ImmTAC to redirect T cells against melanospheres was assessed in vitro using IFN γ and granzyme B Elispots in the presence or absence of commercially sourced IFN α 2 or IFN β . Patient derived TO were generated by Tempus³ and screened for HLA-A*02:01 and antigen positivity. ImmTAC-mediated killing of relevant TO was assessed by 3D high content imaging.

Results: Melanospheres formed from all melanoma cell lines tested, and upregulated melanin synthesis genes including PMEL, MITF, and MLANA. This translated into visible but heterogenous melanin expression. ImmTAC were capable of redirecting T cells against cells from melanospheres to produce IFN γ (EC₅₀ 23 pM - 4.8 nM) and granzyme B (EC₅₀ 130 pM - 1.4 nM). Treatment of 3D cultures with IFN α 2 or IFN β for 48 hours augmented ImmTAC-mediated redirection of IFN γ secretion by a mean of 4.42-fold. To further assess ImmTAC-mediated T cell redirection against more complex in vitro cultures, TO were derived from patient tumours and co-cultured with PBMC for 96 hours. In these settings, clinically relevant doses of ImmTAC redirected T cells to induce cell lysis of antigen positive TO (EC₅₀ 29.3 pM).

Conclusions: 3D melanospheres and TO may provide useful models to study tumour biology, heterogeneity, and ImmTAC activity. ImmTAC were capable of redirecting T cells against these in vitro tumour models, which will allow for better understanding of mechanism of action. 1. Nathan P et al. *N Engl J Med.* 2021. 1196-1206. 2. Larsen B et al. *Cell Rep.* 2021; 36(4).

Legal entity responsible for the study: The authors.

Funding: Immunocore Ltd.

Disclosure: All authors have declared no conflicts of interest.

<https://doi.org/10.1016/j.annonc.2022.07.1770>

1693P Incidence of NTRK genes fusion in adult brain tumours: A prospective cohort of 140 patients with cerebral gliomas and brain metastases

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Background: Oncogenic fusion of neurotrophin receptor tyrosine kinase *NTRK1*, *NTRK2*, or *NTRK3* genes have been found in different types of solid tumours. Nevertheless, the incidence of *NTRK* gene fusions in these cancers remains quite unexplored. The treatment of patients with TRK fusion cancer with a first-generation TRK inhibitor (such as larotrectinib or entrectinib) is associated with high response rates (>75%), regardless of tumour histology and presence of metastases, and acceptable toxicity profile. Due to the efficacy of TRK inhibitor therapy and the recent Food and Drug Administration and European Medicines Agency approval of larotrectinib and entrectinib, it is clinically important to identify patients accurately and efficiently with TRK fusion cancer. In this study, we provide unique data on the incidence of oncogenic *NTRK* gene fusions in adult patients with brain metastases (BM) and gliomas.

Methods: In this study, 140 samples fixed and paraffin-embedded tissue (FFPE) of adult patients with gliomas (60 whose 19 of WHO grade II, 21 of WHO grade III and 20 glioblastomas) and BM (80 with different primary tumours) are analysed. Identification of *NTRK* gene fusions is performed using next-generation sequencing (NGS) technology on the Ion Torrent S5XL automaton with the Oncomine Focus RNA assay kit (ThermoFisher). The analysis is carried out using the Ion Reporter software. A minimum of 50,000 mapped reads is required to allow interpretation of the result.

Results: Among the first 47 samples (30 gliomas and 17 BM) analysed, a *NTRK3* gene fusion was identified in a WHO grade II glioma sample. Another 93 samples are to be analysed. These are composed of 63 BM and 30 gliomas.

Conclusions: Due to the size of the population analysed, this study will provide pioneering data on the incidence of *NTRK* gene fusions in brain tumours, which could strongly support the relevance of innovative clinical trials with specific targeted therapies (larotrectinib, entrectinib) in this patients's population.

Legal entity responsible for the study: Groupement de Coopération Sanitaire Ramsay Santé pour l'Enseignement et la Recherche.

Funding: Bayer HealthCare SAS-Pharmaceuticals.

Disclosure: All authors have declared no conflicts of interest.

<https://doi.org/10.1016/j.annonc.2022.07.1771>

1694P Single cell characterization of longitudinal biopsies from breast cancer patients treated with the aromatase inhibitor letrozole and the CDK4/6 inhibitor ribociclib

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Background: The recent introduction of CDK4/6 inhibitors has been one of the most pivotal breakthroughs in breast cancer therapy in the last decades. A growing body of evidence proposes that CDK4/6 inhibitors potentially influence the recruitment of immune cells in the tumor microenvironment, but also induce a senescent-like phenotype in the tumor cells. The NEOLETRIB-study is a multicenter, single-arm, open-label, neoadjuvant, phase II trial. Locally advanced breast cancer patients belonging to the luminal-A and luminal-B subtypes, received neoadjuvant therapy for 6 months with ribociclib (600 mg daily, 21 days on / 7 days off) and letrozole (2.5 mg daily).

Methods: Pre-treatment, on-treatment (end of the first 21 days of therapy) and end-of-treatment biopsies were subjected to single-cell transcriptome using the Chromium Single-Cell v2 5' Chemistry. Raw gene expression matrices were analyzed with the Seurat package (v4.0.2). First, empty droplets and droplets containing two cells were filtered out using the EmptyDrops and scDblFinder algorithms respectively. In addition, cell barcodes with < 500 UMIs, < 300 expressed genes, > 5000 expressed genes or > 15% of reads mapping to mitochondrial RNA were filtered out. The gene expression of the remaining good quality cells was normalized.

Results: Variably expressed genes were used to construct principal components (PCs). Significant PCs were further used to cluster cells. We identified 8 main cell types: T