

correlate with survival outcomes. ECOG0 patients had a median survival of 146.7 weeks, compared to ECOG1 patients, which had a median survival of 86.6 weeks (hazard ratio: 0.35; 95% CI: 0.14-0.84; P = 0.033).

**Conclusions** Even though ECOG performance status is found to be statistically associated with survival outcome of patients with advance stage NPC. This result is unsurprising as the prognostic value of ECOG has been well documented in literature, albeit in other cancer types. Other patient parameters such as gender, age, initial stage of cancer, NLR, and initial EBV titre, did not yield significance and did not prognosticate for survival outcome. This finding supports our hypothesis that the improved survival outcomes observed in advance NPC patients treated with chemotherapy followed by EBV CTL-immunotherapy is due to effects of treatment and not because of intrinsic patient factors.

#### REFERENCE

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#### NOVEL INSIGHTS INTO IMMUNE-INDEPENDENT FUNCTIONS OF IMMUNE CHECKPOINT INHIBITORS IN OESOPHAGEAL ADENOCARCINOMA; POTENTIAL IMPLICATIONS FOR DESIGNING COMBINATION IMMUNO-CHEMOTHERAPY REGIMENS TO ACHIEVE SYNERGISTIC RESPONSES

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**Background** Immune checkpoint inhibitors (ICIs) reinvigorate anti-tumour immunity in oesophageal adenocarcinoma (OAC). However, emerging studies have identified novel immune-independent functions for immune checkpoints (ICs) in other solid tumour-types, whereby IC-intrinsic signalling in gastric cancer cells confers chemoresistance. This study explores immune-independent functions of ICs in OAC and if therapeutic blockade may enhance chemotherapy toxicity.

**Materials and Methods** OAC cells were screened *in vitro* and *in vivo* ( $n=14$  OAC human tissue biopsies) for a range of ICs (PD-1, TIGIT, TIM-3, LAG-3, A2aR, PD-L1, PD-L2, CD160) by

flow cytometry. The phenotype of OAC cells expressing ICs was also assessed for features of stemness (ALDH, CD54), senescence ( $\beta$ -galactosidase) and invasiveness (vimentin) in the absence and presence of chemotherapy by flow cytometry. OAC cells were also treated with chemotherapy in the absence and presence of a MEK inhibitor to determine if MEK signalling regulated IC expression. Importantly, the effect of ICIs on the hallmarks of cancer in OAC cells was assessed which included: OAC cell viability (CCK-8 assay and western blot to assess Bcl-xL and Bcl-2 levels), proliferation (BrdU assay and ki67 expression by intracellular flow cytometry), chemo-sensitivity (annexin-V propidium iodide assay and cell cycle analysis by flow cytometry and expression of chemotherapy efflux and influx pumps by western blot: ATP7a, ATP7b, CTR1 and ABCB9), metabolism (seahorse), invasiveness and stemness characteristics (vimentin and aldefluor assay, respectively by flow cytometry) and DNA repair ( $\gamma$ H2ax by flow cytometry) to assess levels of DNA repair and the expression of DNA repair genes were quantified by qPCR: MLH1, SMUG1, PARP1, MMS19) was assessed in OAC cells.

**Results** A subpopulation of stem-like, senescent and vimentin<sup>+</sup> OAC cells were enriched for ICs, which was enhanced by FLOT and CROSS chemotherapy regimens. IC expression increased on the surface of OAC cells 48h post-chemotherapy treatment and was sustained up to 3 weeks post-treatment *in vitro*. Inhibition of pro-survival MEK signalling reduced chemotherapy-induced upregulation of ICs. Blockade of PD-1, TIGIT, A2aR, TIM-3 and PD-L1 decreased proliferation, DNA repair, induced apoptosis and enhanced toxicity of FLOT in OAC cells. Blockade of TIGIT decreased pro-survival Bcl-xL factor, induced cell death and promoted a more glycolytic phenotype in OAC cells.

**Conclusions** Several novel ICs have been identified as potential targets to enhance chemotherapy efficacy in OAC. Upregulation of ICs on OAC cells following chemotherapy may represent potential mechanisms of chemo-immune resistance for stem-like, senescent and vimentin<sup>+</sup> aggressive cancer cell clones. Combining ICIs with chemotherapy may synergise with chemotherapy in OAC patients via immune-independent mechanisms and boost response rates to current standards of care. Further studies are warranted through clinical trials to further establish synergistic ICI-chemotherapy combinations in OAC.

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