

P09.05 PLASMA CD27, A SURROGATE OF INTRATUMORAL CD27-CD70 INTERACTION, CORRELATES WITH IMMUNOTHERAPY RESISTANCE IN RENAL CANCER

^{1,2}N Benhamouda, ¹I Sam*, ¹N Epailard, ^{1,2}A Gey, ^{1,2}A Saldmann, ^{1,2}J Pineau, ³M Hasan, ⁴V Verkarre, ³V Libri, ^{3,5}S Mella, ^{1,2}C Granier, ⁴C Broudin, ⁵P Ravel, ^{3,5}B Jabla, ⁶N Chaput, ⁷L Albiges, ^{8,9}Y Vano, ¹⁰O Adotevi, ^{1,8}S Oudard, ^{1,2}E Tartour. ¹INSERM U970 PARCC Université de Paris, Paris, France; ²Service d'Immunologie Biologique, APHP, Hôpital Européen Georges Pompidou (HEGP), Paris, France; ³Cytometry and Biomarkers UTechS, Center for Translational Science, Institut Pasteur, Paris, France; ⁴Department of Pathology, APHP, HEGP, Paris, France; ⁵IRCM – INSERM U1194, Institut de Recherche en Cancérologie de Montpellier, Montpellier, France; ⁶Université Paris-Saclay. Laboratory of Immunomonitoring in oncology, Institut Gustave Roussy, Paris, France; ⁷Université Paris-Saclay. Department of Medical Oncology. Institut Gustave Roussy, Paris, France; ⁸Department of Medical Oncology, HEGP, Paris, France; ⁹Centre de Recherche des Cordeliers, INSERM UMRS1138, Université de Paris, Paris, France; ¹⁰University Bourgogne Franche-Comté, INSERM, EFS BFC, UMR1098; INSERM CIC1431, Department of Pneumology, University Hospital of Besançon, Besançon, France

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Background CD70, a costimulatory molecule on antigen presenting cells, is known to activate CD27-expressing T cells. CD27-CD70 interaction leads to the release of soluble CD27 (sCD27). However, persistent interaction of CD27 and CD70 such as in chronic infection may exhaust the T cell pool and promote apoptosis. Surprisingly, our analysis based on TCGA database show that clear cell renal cell carcinoma (ccRCC) expresses the highest levels of CD70 among all solid tumors. Despite the important clinical efficacy of immunotherapy by anti-PD-1 in RCC patients, the overall response to anti-PD1 remains modest. The relationship between the CD27-CD70 interaction in the RCC and the response to immunotherapy is still unclear.

Materials and Methods To study the CD27 and CD70 expression in the tumor microenvironment (TME), FFPE tumor tissues from 25 RCC patients were analysed using multiplex *in situ* immunofluorescence. 10 fresh RCC tumor samples were collected to analyse the phenotype of CD27⁺ T cells by flow cytometry and 4 samples were proceeded for single-cell RNA-seq analysis. A cohort of metastatic RCC patients (n = 35) treated by anti-PD-1 were enrolled for the measurement of plasma sCD27 by ELISA and the survival analysis is also realized.

Results In the TME, we demonstrated that CD27⁺ T cells interact with CD70-expressing tumor cells. In fresh tumors from RCC patients, CD27⁺ T cells express higher levels of cleaved caspase 3 (a classical marker of apoptosis) than CD27⁻ T cells. We confirmed the apoptotic signature (BAX, FASLG, BCL2L11, CYCS, FBXO32, LGALS1, PIK3R1, TERF1, TXNIP, CDKN2A) of CD27⁺ T cells by single-cell RNAseq analysis. CD27⁺ T cells also had a tissue resident memory T cell phenotype with enriched gene expression of ITGAE, PRDM1, RBPJ and ZNF683. Moreover, CD27⁺ T cells display an exhaustion phenotype with the expression of multiple inhibitory receptors gene signature (PDCD1, CTLA4, HAVCR2, LAG3, etc). Besides, intratumoral CD27-CD70 interaction significantly correlates with plasma sCD27 concentration in RCC (p = 0.0017). In metastatic RCC patients treated with anti-PD-1, higher levels of sCD27 predict poor overall survival (p = 0.037), while it did not correlate with inflammatory markers or clinical prognostic criteria.

Conclusions In conclusion, we demonstrated that sCD27, a surrogate of T cell dysfunction in tumors likely induced by persistent interactions of CD27⁺ T cells and CD70-expressing tumor cells, is a predictive biomarker of resistance to

immunotherapy in mRCC. To our knowledge, this is the first report showing that a peripheral blood biomarker may reflect certain aspects of the tumor-host interaction in the tumor microenvironment. Given the frequent expression of CD70 and CD27 in solid tumors, our findings may be further extended to other types of tumors. CD70-CD27 interaction could thus be considered as a mechanism of tumor escape, but also a novel therapeutic target in cancers.

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P09.06 INVESTIGATING VARIOUS PATIENT PARAMETERS AS PROGNOSTIC MARKERS FOR PATIENTS WITH ADVANCE STAGE NASOPHARYNGEAL CARCINOMA UNDERGOING INDUCTION CHEMOTHERAPY FOLLOWED BY EPSTEIN-BARR VIRUS CYTOTOXIC T-LYMPHOCYTE IMMUNOTHERAPY

¹A Chu*, ²S Han, ²H Toh. ¹Duke-NUS Medical School, Singapore; ²National Cancer Centre, Singapore

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Background Previous prospective phase II study conducted by our research group at the National Cancer Centre Singapore had shown the efficacy of combined induction chemotherapy followed by cytotoxic T-lymphocyte (CTL) immunotherapy as a first-line treatment for advance nasopharyngeal carcinoma (NPC) – i.e. median survival for patients treated with combined therapy was 29.9 months, compared to 17.7 months for patients who received only standard chemotherapy.¹ Using the same data set, we further investigate the correlation between various patient factors (Eastern Cooperative Oncology Group (ECOG) score, gender, age, initial stage of cancer, neutrophil-to-lymphocyte ratio (NLR), initial EBV-DNA titre) on overall survival (OS). This is to further validate our hypothesis that the improved OS is due to an effect of treatment and not due to intrinsic patient factors.

Materials and Methods Survival distribution curves were estimated using the Kaplan-Meier method and differences were compared statistically using log-rank test. IBM SPSS statistics software package (v. 22) was used for the purpose of statistical analysis. Overall survival was defined as time from diagnosis to date of event (date of death/date of last follow-up). For analysis of overall survival, data for patients who were alive or who were lost to follow-up were censored at the end of study period.

Results It was revealed that lower ECOG score, a scale used to assess the physical condition of patients, correlated with longer OS while other characteristics such as gender, age, initial stage of cancer, NLR, and initial EBV-DNA titre did not

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.

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P09.07

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

¹M Davern*, ¹C Buckley, ²C Fitzgerald, ¹AB Heeran, ¹NE Donlon, ¹J McGrath, ¹R O' Brien, ¹F O' Connell, ²B Murphy, ¹N Lynam-Lennon, ¹JV Reynolds, ¹SG Maher, ¹AD Sheppard, ¹A Bhardwaj, ¹A Bhardwaj, ¹C Butler, ¹N Ravi, ¹J Lysaght. ¹Trinity College Dublin, Dublin, Ireland; ²Royal College of Surgeons, Dublin, Ireland

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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 (β -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 (γ 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⁺ 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⁺ 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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