signal in neighbouring T cells, and these molecules will then transmit either PD-1 or LAG-3 signals. To test their expression, human T cells were transduced with lentivectors expressing these constructs singly or in combination and their phenotypes were characterized by flow cytometry analysis.

**Results:** Both constructs showed differential phenotypes and expression levels within different surface and intracellular molecules compared with their WT and CD3 activator controls, such as CD3, CD4, CD27, CD28, CD69, CD62L, CD45RA, PD1, LAG3, TIM-3, CLA-4, KIR2DL1/2/3/5/55, IFN-γ, IP-10/II-12/II-13, IL-4, IL-2 and IL-17A, among others.

**Conclusions:** These results showed that these molecules had functional PD-1 and LAG-3 constitutive inhibitory signalling in T cells leading T cell dysfunctionality. This will allow to study the reasons behind the intrinsic resistance to PD-1 blockade.

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**In vitro analysis of the combination of APR-246 and carboplatin in triple negative breast cancer (TNBC) and high grade serous ovarian cancer (HGSOCC) cell lines and its impact on Aurora kinase A (AURKA)/p35 pathway**

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**Background:** AURKA is a protein that regulates mitotic spindle formation and p53 is involved in cell cycle regulation. p53 mutations and AURKA overexpression is a frequent alteration in both TNBC and HGSOCC cell lines which lead to carboplatin sensitivity. APR-246 is a new targeted agent that modulates abnormal p53 in mutated cells. The aim of our study was to assess the effect of the combination of carboplatin (carbo) plus APR-246 in TNBC and HGSOCC cell lines.

**Methods:** We selected two TNBC (MDA-MB-231 and MDA-MB-436) and one HGSOCC cell line (Kuramochi). Next-generation sequencing was performed in order to assess more relevant mutations. MTT experiments were performed to calculate the IC50 for APR-246 and carbo. For the combination (combo) increasing IC50 of either APR-246 or carbo was assessed in the presence of a constant concentration of each agent. Western-blot (WB) and PCR analysis was performed in each line after exposure to APR-246, carbo or the combination to determine expression of AURKA and p53.

**Results:** The mutation profile of each line and the IC50 of APR-246, carbo and combo is shown in the table.

**Table: 18P**

<table>
<thead>
<tr>
<th>P53 - IC50 BRCa carbo</th>
<th>IC50 carbo in Combo (APR-246 constant)</th>
<th>IC50 APR-246 P value in combo (carbo constant)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA-MB-231 Mut - 242.6 uM</td>
<td>0.0001 41.77 uM</td>
<td>22.37 12.19 &lt;0.0001</td>
</tr>
<tr>
<td>MDA-MB-436 WT - 38.73 uM</td>
<td>0.0001 5.01 uM</td>
<td>8.85 1 &lt;0.0001</td>
</tr>
<tr>
<td>Kuramochi Mut - 35.86 uM</td>
<td>0.0001 18.86 uM</td>
<td>21.78 13.97 &lt;0.0001</td>
</tr>
</tbody>
</table>

Our results showed that IC50 of carbo and APR-246 decreased when administered in combination with constant doses of each other regardless of the subtype (TNBC vs HGSOCC) and p53 mutations but more evident in the MDA-MB-231 BRCaWt cell line. PCR in MDA-MB-231 and MDA-MB-436 showed that AURKA was overexpressed by exposure to APR-246 and carbo. For the combination (combo) increasing IC50 of either APR-246 or carbo was upregulated after exposure to both (carbo lines) or combo (only 436). Kuramochi cells showed an underexpression of AURKA after combo and an upregulation of p53 after carbo or combo. Similar results were shown with WB in these cell lines.

**Conclusions:** Addition of APR246 to carboplatin increased apoptosis in both TNBC and HGSOCC cell lines regardless of p53 mutations status in this in vitro study. AURKA and p53 expression was modified after exposure to carbo or combo.