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EACR 2023 Congress Abstracts

Contents

Proffered Papers

10-minute talks awarded for the highest scored abstracts, embedded in the scientific symposia sessions. These presentations are not accompanied by a poster.

Posters in the Spotlight

Tuesday 13 June, 17:30 - 18:30, Poster and Exhibition Hall
Wednesday 14 June, 17:15 - 18:15, Poster and Exhibition Hall

Dedicated sessions taking place in the spotlight area within the Poster and Exhibition Hall. Poster presenters with high scoring abstracts will give short presentations of up to 10 minutes. Their posters will also be available to view during the Poster Discussion Sessions.

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Cell viability assays showed that AM combination with conventional chemotherapy significantly increases their cytotoxic effect. The analysis of this data with CompuSyn Software revealed synergy of the drugs, especially in the combination of AM with cisplatin. According to flow cytometry and western blot results, AM is able to enhance apoptosis in cisplatin-treated cells.
Therapeutic in vivo analyses showed significant impairment in tumor growth in mice treated with AM and cisplatin combination.**Conclusion**
Our results demonstrate there is a sensitizing effect when combining AM, a novel specific direct-binding survivin inhibitor, with conventional chemotherapy, especially cisplatin.
Funding:Instituto Salud Carlos III (PI18/00441;PI22/00256),Junta de Castilla y León (BU067P20) and ERDF. We thank CERCA Programme/Generalitat de Catalunya for institutional support.

EACR23-0460
Overexpression of CDK2 and CCNE1 associate with sensitivity to the Wee1 inhibitor adavosertib in primary cultures

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Introduction
Cyclin E1 (*CCNE1*) amplification has been associated with response to WEE1 G2 checkpoint kinase inhibition (Wee1) but the role of tumor protein p53 (*TP53*) mutations and cyclin dependent kinase 2 (*CDK2*) upregulation has not been clarified. Here, we analyzed *CCNE1* and *CDK2* levels, *TP53* mutations and sensitivity to adavosertib in primary cultures derived from lung and ovarian cancer patients.
Material and Methods
The levels of *CDK2* and *CCNE1* mRNA in lung (n = 14) and ovary (n = 3) primary cultures derived from pleural effusions and ascites were measured using a massive hybridization 770 mRNA probe commercial panel (IO360, NanoString® Technologies), while *TP53* mutation status was determined by NGS. The cut-off for high expression was established as the geomean plus the standard deviation of all samples. Five primary cultures with different *CDK2* and *CCNE1* mRNA levels and *TP53* status were treated with adavosertib.
Results and Discussions
Among the 17 samples analyzed by massive hybridization, an ovarian primary culture showed high mRNA levels of *CCNE1* while another ovarian and a lung primary presented high *CDK2* expression. The three primaries were treated with adavosertib, together with two additional lung primary cultures with low *CDK2* and *CCNE1*. The three cultures overexpressing *CDK2* or *CCNE1* were moderately sensitive to the Wee1 inhibitor, with half-maximal inhibitory concentrations (IC50s) 200-500 nM. In contrast, the two low expressing primaries were completely resistant to the drug, with IC50s > 10 μM. Regarding *TP53* status, one of the primaries was wild-type while the other four harbored loss-of-function mutations.
Conclusion
Elevated levels of *CDK2* and *CCNE1* mRNA expression associate with sensitivity to Wee1 inhibition in lung and ovarian primary cultures.

EACR23-0465
Preclinical validation of rilmenidine for repurposing in pancreatic ductal adenocarcinoma

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Introduction
Pancreatic ductal adenocarcinoma (PDAC) has dismal prognosis, as there are no screening tests available, most often is diagnosed in the metastatic phase of the disease and is refractory to conventional, targeted and immunotherapy. We have examined the expression and role of the novel tumor suppressor nischarin (NISCH) in PDAC and the effects of treatment with the agonist rilmenidine (approved for treatment of hypertension) in order to determine the potential of nischarin agonists for repurposing in this deadly disease.
Material and Methods
Nischarin expression was examined by immunohistochemistry in PDAC tissue array. NISCH was knocked-down (KD) in two PDAC cancer cell lines and the effects were examined by transcriptome sequencing and gene set enrichment analysis. The effects of rilmenidine treatment on pancreatic cancer cells were examined in assays for proliferation, migration and invasion *in vitro* and in the Tg(fli1:EGFP) zebrafish model. Effect of rilmenidine treatment on cancer cell-cancer associated fibroblast (CAF) co-cultures was analyzed with dot blot cytokine array and growth factor qRT-PCR array. Ultimately, the effect of rilmenidine on the ex vivo PDAC tumor cultures was examined by western blot.
Results and Discussions
Nischarin was expressed in both the tumor and the stromal compartment of PDAC. In cancer cells NISCH KD induced changes associated with cell adhesion and vesicular transport. Treatment with rilmenidine *in vitro* did not decrease cancer cell viability at concentrations achievable in patients, but significantly decreased cancer cell adhesion, migration and cell invasion through matrices. In the zebrafish model, rilmenidine drastically reduced tumor invasion. In cancer cell-CAF co-cultures rilmenidine reduced production of pro-inflammatory cytokines. Ultimately, in the *ex vivo*patient tissues treatment with rilmenidine remodeled extracellular matrix.
Conclusion

Taken together, nischarin agonist rilmenidine reduces PDAC cancer cell invasion *in vitro* and *in vivo* and has an impact on cancer-stroma interactions. Our study lays a ground for potential repurposing of antihypertensive drug rilmenidine as antimetastatic therapeutic in PDAC.

EACR23-0478
Lunatin-1: A scorpion venom peptide that induces instant cell death by necrosis in the breast cancer cell line MDA-231

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Introduction
Previous studies from our group have shown that Lunatin-1, a peptide isolated from the *Hadruroides lunatus* scorpion venom, induce apoptosis in human promyelocytic leukemia HL-60 cell line and causes death in MCF-7 and MDA-231 human metastatic cancer cells lines by unknown mechanisms.

Material and Methods
Synthetic Lunatin-1 was purified by high-performance liquid chromatography (HPLC) and analyzed by mass spectrometry (MALDI-TOF/TOF). We conducted a similarity search of Lunatin-1 against human proteins using Blast tool and performed gene ontology analysis with David tool. To determine the IC50 of Lunatina-1 on MDA-231 cell line, we treated the cells with different concentrations of Lunatin-1 and evaluated cell viability using resazurin. We also treated the cells with 25 μM of Lunatin-1 to evaluate cell viability by measuring propidium iodide (PI) stain, and its morphology during 1h using a Cell Imaging Multimode Reader (Cytation).

Results and Discussions
Lunatin-1 induced cytotoxicity in MDA-MB-231 cell line with an experimental IC50 of 31.25 μM. Treatment with 25 μM of Lunatin-1 induced necrosis, as evidenced by the presence of IP positive-stained cells after 20 minutes ($p< 0.05$) compared to vehicle (DMSO 0.5%). Moreover, cell swelling was observed after 5 minutes of treatment ($p<0.05$). Instant cell death was also observed ($p<0.05$) when compared with untreated cells. Bioinformatics showed that Lunatin-1 has a high degree of similarity with transport membrane proteins. This, together with the observed rapid effects suggested us that Lunatina-1 may impair electrolytic cell homeostasis by binding to transport membrane proteins.

Conclusion
Lunatin-1 induces death of breast cancer cell line and may be used as an antitumoral drug lead.

EACR23-0487
CBFβ inhibitors suppress myeloma cell growth and invasion by targeting of both Runx1 and Runx2 in myeloma cells

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Introduction
Multiple myeloma (MM) is a plasma-cell malignancy. The aggressiveness of MM cells is governed by a complicated network of molecular signals. The coordination of multiple genes involved in the network of these molecular signals in MM cells may be under the control of a few transcription factors. Using raw data extracted from GEO datasets (accession number GSE6477), we found a significant increase in the expression of Runt-related transcription factor factor (Runx) 1 (Runx1) and 2 (Runx2) in MM cells of MM patients, compared with healthy donors. Both of Runx1 and Runx2 forms a heterodimeric complex with core-binding factor β subunit (CBFβ). CBFβ enhances the affinity of Runx proteins for DNA binding and protects them from proteasome-mediated degradation. In current study, we investigated (1) whether knockdown of Runx1 or Runx2 in MM cells inhibits MM progression, and (2) whether CBFβ inhibitor(s) suppress MM growth and invasion by targeting of both Runx1 and Runx2 in MM cells.

Material and Methods
Runx1 or Runx2 expression was knocked down in murine 5TGM1 MM cells by transduction with specific Runx1 or Runx2 shRNA lentiviruses or non-targeted (NT) shRNA control (Sigma). NT control or Runx1 knockdown (k/d) or Runx2- k/d 5TGM1 cells were injected into 6 week old C57BL/KaLwRij mice via the tail vein (10^6 cells/injection). Serum IgG2bκ levels (a soluble marker of 5TGM1 cells) were measured bi-weekly by ELISA. Small molecule inhibitors of CBFβ, AI-10-104 and AI-14-91, and a control compound, Al-4-88, were obtained from Dr. Bushweller's lab (University of Virginia, USA).

Results and Discussions
Our in vivo studies demonstrated that knockdown of either Runx1 or Runx2 in 5TGM1 MM cells significantly inhibited tumor growth in syngenic C57BL/KaLwRij mice, compared to NT-control 5TGM1 cells. Next, 5TGM1 murine MM cells and CAG human MM cells were treated with CBFβ inhibitors AI-10-104 or AI-14-91 or control compound Al-4-88 (40 μm) for 24, 48, 72h respectively. Real-time PCR and western blot showed significantly reduced levels of both Runx2 and Runx1 in the nucleus of AI-10-104 or AI-14-91 treated MM cells. MTT and invasion assays showed that the proliferation and invasion of both 5TGM1 and CAG MM cells were significantly inhibited by AI-10-104 and AI-14-91 (40 μM), as compared with Al-4-88 control.

Conclusion
our study uncovered novel roles of MM cell-expressed Runx1 and Runx2 in MM progression, and identified novel targets (MM cell-expressed Runx1 and Runx2) and new drugs (CBFβ inhibitors) for MM treatment.

EACR23-0522
Benefits of cannabidiol as an anti-breast cancer agent when combined with Exemestane