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Background: Typical and atypical bronchial carcinoids (TBC and ABC) display a wide range of clinical presentations and may behave very differently. Survival prognostic markers are necessary to better define therapeutic strategies. AIM: verify that the NEP-Score, recently proposed as prognostic score, can be applied in a homogeneous TBC and ABC cohort and identify a derivate prognostic marker taking into account clinical and pathological characteristics at diagnosis. **Methods:** Age, site of primary tumor, primary tumor surgery, symptoms, Ki67, timing of metastases of 64 patients including TBC and ABC were evaluated to calculate the NEP-Score at the end of follow-up (NEP-T). We then assessed a derivative score considering the NEP-Score at diagnosis (NEP-D): this score does not consider the appearance of new metastases during follow-up. We then considered the patients that were alive or dead at the end of follow-up (EOF). A NEP-Score threshold to predict survival was investigated. **Results:** live patients at EOF displayed a mean NEP-T and mean NEP-D significantly lower as compared to those that were dead. A NEP-T threshold >138 significantly predicts survival. ABC relapsed more frequently as compared to TBC. Male gender as well as previous malignancy were negative prognostic factors for survival. **Conclusions:** We found that NEP-Score is applicable to a series of bronchial neuroendocrine neoplasms. In addition, we propose NEP-D as a simple, quick and cheap prognostic score that can help clinicians in decision making. Moreover, the use of a NEP-D threshold can predict NEN aggressiveness and may be used to define the best personalized therapeutic strategy. Furthermore we found additional prognostic factors that together with the NEP-Score could improve prognosis evaluation at diagnosis by using easily accessible information.

Tumor Biology

HORMONE ACTIONS IN TUMOR BIOLOGY: FROM NEW MECHANISMS TO THERAPY

Evaluation of the Octreotide Acetate Pen Injector in a Formative Human Factors Study

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Introduction: Subcutaneous injection of octreotide acetate is indicated to treat adults with acromegaly and diarrhea associated with carcinoid tumors or vasoactive intestinal peptide tumors. In this formative human factors study, we evaluated the readability and comprehension of the instructions for use (IFU) and ease of use of the octreotide pen injector. **Methods:** The study enrolled patients and healthcare practitioners who would be using the pen injector. The IFU contained a stepwise process with illustrations to detail injection administration and safe storage of the octreotide pen injector. Participants read the IFU and familiarized themselves with the device. Participants administered 2 unaided injections into skin-like pads. Injection success was defined as an attempt that delivered the correct dose into the pad. Each injection was evaluated by objective performance and subjective measures. Objective performance measures included assessment of steps necessary to deliver the correct medication dose and ensure user safety. Subjective measures included soliciting participant feedback on perceived success and difficulties administering a dose with the octreotide pen injector, as well as suggestions for improvements. Additional goals included evaluation of the IFU and octreotide pen injector usability aspects. **Results:** A total of 8 patients and 3 healthcare practitioners enrolled in the study. All (n = 11) participants successfully administered both injections, leading to an overall injection success rate of 100% across twenty-two injections. Subtask errors included participants priming the pen injector with the incorrect dose (n = 1) and not holding the injection button for 10 seconds after the injection (n = 2), but neither error resulted in dosing failure. Participant suggestions for improving the IFU included changes to the illustration of the plunger, reordering statements to clarify the priming process, and detailing how long to let the pen injector come to room temperature. **Conclusion:** Overall, participants felt the octreotide pen injector was easy to use and the instructions were clearly written and illustrated. Participant feedback and observations by moderators of the study led to recommendations for improvements to the clarity of the IFU.

Tumor Biology

HORMONE ACTIONS IN TUMOR BIOLOGY: FROM NEW MECHANISMS TO THERAPY

FOXO1 Mitigation of FOXL2C143W/SMAD3 Transcriptomic Landscape in a Model of Granulosa Cell Tumor.

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Background: Adult granulosa cell tumor (aGCT) is a rare type of stromal cell malignant cancer of the ovary. Postmenopausal genital bleeding is the main aGCT clinical sign which is attributed to estrogen excess driven by CYP19 upregulation. Typically, aGCTs that are diagnosed at an initial stage can be treated with surgery. However, recurrences are mostly fatal[1]. Current studies are focused on finding new molecular markers and targets that aim to treat the aGCTs recurrence. Between 95-97% of aGCTs harbor a somatic mutation in the FOXL2 gene, Cys134Trp (c.402C<G)[2]. A TGF-β pathway protein, SMAD3, was

identified as an essential partner in FOXL2^{C134W} transcriptional activity driving CYP19 upregulation[3]. Recently, the antitumoral FOXO1 gene has been recognized as a potential target for suppressing the FOXL2^{C134W} pathogenic action[4]. **Aim:** The objective of this study was to examine whether FOXO1 upregulation affects the FOXL2^{C143W}/SMAD3 transcriptomic landscape. **Methods:** RNA-seq analysis was performed comparing the effect of FOXL2^{WT}/SMAD3 and FOXL2^{C143W}/SMAD3 overexpression in presence of FOXO1 by transfection of an established human GC line (HGrC1). RNA-seq libraries were prepared using the illumina TrueSeq and sequenced using an illumina HiSeq Platform4000. To quantify transcript abundance for each sample we used salmon (1.1.0) with default parameters, using indexes from hg38. Data was subsequently imported in R using the tximport package and processed with the DESeq2 package. **Results:** RNA-seq data show that FOXL2^{C143W}/SMAD3 significantly drives 717 genes compared with the WT and enabled us to identify targets (TGFB2, SMARCA4, HSPG2, MKI67, NFKBIA) and neoplastic pathways directly associated with the mutant. To provide evidence that the differences in gene expression were attributed to a direct consequence of FOXL2 binding, we annotated gene promoters with previously published FOXL2 ChIP-seq analysis. The majority (73-40%) of the differential expressed genes (DEGs) between FOXL2^{C134W} and FOXL2^{WT} had a FOXL2 binding site at their promoters, which was a significantly higher proportion than in non-DEGs (Fisher's exact test, murine: p= 7.9x10^{-157}; human, p= 9.9x10^{-39}). Surprisingly, the number of DEGs between FOXL2^{C134W} + FOXO1 and FOXL2^{WT} was much lower (230) with respect to the number of DEGs between FOXL2^{C134W} and FOXL2^{WT} (717, of which 130 in common; linear regression slope ß = 0 .58), suggesting that the effect of FOXL2^{C134W} compared with FOXL2^{WT} is moderated by the addition of FOXO1. **Conclusions:** Our transcriptomic study provides the first evidence that FOXO1 can efficiently mitigate 40% of the altered genome-wide effect specifically related to FOXL2^{C134W} in a model of human aGCT.1 Farkkila, A. *et al. Ann Med* (2017). 2 Jamieson, S. & Fuller, P. J. *Endocr Rev* (2012). 3 Belli, M. *et al. Endocrinology* (2018). 4 Belli, M et al. *J Endocr Soc* (2019).

Tumor Biology

HORMONE ACTIONS IN TUMOR BIOLOGY: FROM NEW MECHANISMS TO THERAPY

Functional Characterization of the Orphan Nuclear Receptor TLX in Triple Negative Breast Cancer

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Despite the development of various therapeutic strategies, breast cancer persists as the second leading cause of cancer-related death among women in the United States. While endocrine modulation and monoclonal antibody therapy have proved to be indispensable modes of intervention for hormone receptor (HR)-positive and HER-2 positive patients, the triple negative breast cancer (TNBC) patient population do not respond to these therapies. As TNBC is considered one of the most challenging subtypes of breast cancer to treat, there is a significant need for the development of targeted therapeutics. Due to their well-known amenability to small-molecule modulation, we investigated whether any nuclear receptors beyond those that are traditionally studied in breast cancer (e.g. ER, PR, and AR), may represent a novel target in the TNBC patient population. Analysis of clinical data revealed that expression of the orphan nuclear receptor TLX (NR2E1) was positively correlated with relapse-free survival, distant metastasis-free survival, and overall survival in both ER-negative and basal-like breast cancer patients. **Therefore, we hypothesized that TLX could influence the pathophysiology of TNBC**. To interrogate this hypothesis, we established TNBC cells with stable expression of TLX in order to identify direct regulatory targets, as well as the precise physiological mechanism(s) TLX may be regulating. To date, our work has revealed that TLX inhibits proliferation, slows migration, alters chemosensitivity, and impairs cell cycle progression in TNBC cells. In agreement with these findings, our work has also revealed that TLX is capable of modulating the expression of several genes that are known to regulate the processes of growth, migration, and cell cycle. Taken together, our early work supports our hypothesis, and provides valuable insight into the potential pro-survival function of TLX in TNBC. Ongoing work will continue to probe the mechanisms by which TLX impacts breast cancer biology, and establish whether the growth-inhibitory effects translate to *in vivo* models. As prior work has demonstrated that TLX's transcriptional activity can be regulated by both synthetic and natural ligands, the results of our work would provide the foundational data necessary for the development of a TLX-based therapy for a patient population with limited therapeutic options and a poor prognostic outlook.

Tumor Biology

HORMONE ACTIONS IN TUMOR BIOLOGY: FROM NEW MECHANISMS TO THERAPY

Growth Hormone Receptor Inhibition Sensitizes Human Pancreatic Cancer to Chemotherapy Treatments

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Human growth hormone (GH) and its cognate growth hormone receptor (GHR) have been established to have a distinct role in promoting the progression of several types of human cancers. We had earlier described a newfound role of the GH-GHR axis in driving chemoresistance in melanoma by upregulating drug efflux by ABC multidrug transporter expression and a phenotype switch by induction of epithelial-to-mesenchymal transition (EMT). Here we present an in-depth analysis of this role of GH-GHR in the highly therapy resistant human pancreatic cancer which