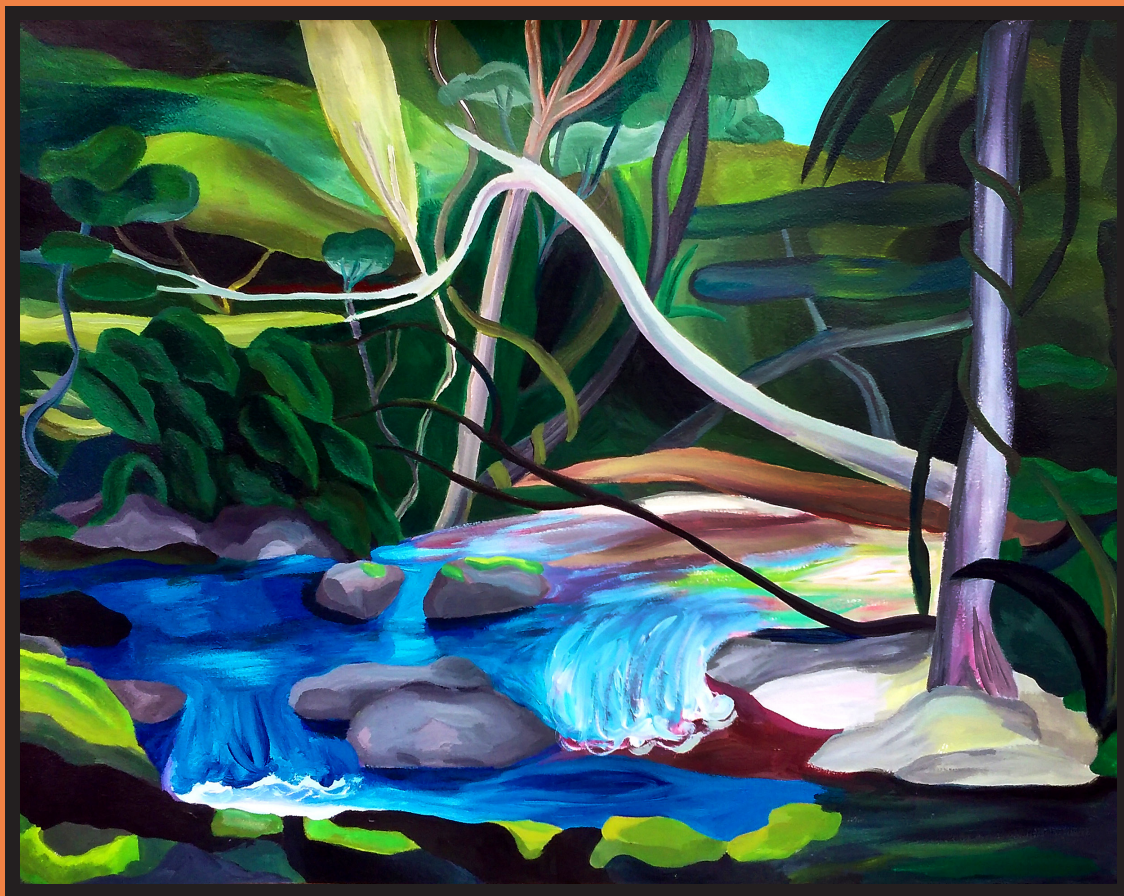


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317. 90. EFFECT OF OLIGO-FUCOIDAN PREBIOTIC AGENT AND BORON NEUTRON CAPTURE THERAPY (BNCT) ON MICROBIOTA IN AN EXPERIMENTAL ORAL CANCER MODEL

M.A. Palmieri¹, S.I. Nemirovsky^{2,3}, I.E. Czornenki⁴, J.A. Goldfinger⁴, P.S. Ramos⁴, E.C.C. Pozzi⁴, S. Thorp⁴, P. Curotto⁴, G.G. Agüero⁴, V.A. Trivillin^{2,4}, M.A. Garabalino⁴, V.A. Medina^{2,5}, C. Costa⁴, A.E. Schwint^{2,4}, M. Pezzoni^{2,4}, A. Monti Hughes^{2,4}

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Boron Neutron Capture Therapy (BNCT) is particle radiotherapy, based on the administration of boron carriers incorporated preferentially by tumor cells, followed by neutron irradiation. BNCT clinical results for Head and Neck cancer have shown significant therapeutic efficacy. Oral microbiota is a heterogeneous group of microbial species colonizing the surfaces of the oral cavity. Microbiota could affect cancer therapy outcomes. Oligo-Fucoidan, isolated from *Laminaria japonica* brown seaweed, is considered a prebiotic agent as it stimulates beneficial bacteria in the gut. Our group showed, in the hamster cheek pouch oral cancer model, an enhancement in tumor control from 67% for BNCT to 94% for BNCT combined with Oligo-Fucoidan. Studies performed by our group described microbiota composition and proportion in a normal and cancerized hamster. In this study, we evaluated if Oligo-Fucoidan and/or BNCT are capable of changing microbiota composition and proportion in tumor-bearing hamsters. Samples of microbiota from the tumor, precancerous surrounding tissue and normal tissue were taken. DNA was extracted and bacterial diversity and taxonomic abundances were characterized by sequencing the 16S rRNA gene. During the cancerization process, we observed that Proteobacteria, Firmicutes and Actinobacteria decreased, while Bacteroidia and Fusobacteria increased (both previously reported in oral cancer patients). Preliminary studies showed that Oligo-fucoidan treatment increased Proteobacteria 4 times ($p < 0.01$) and reduced Bacteroidia 1.4 times ($p < 0.05$). Besides, Oligo-fucoidan tended to reduce Fusobacteria in cancerized pouches. The effect of BNCT on oral microbiota is currently under evaluation. These preliminary results showed that Oligo-fucoidan modulates cancerized hamster cheek pouch microbiota. Acknowledgments: Hi-Q Marine Biotech (Taiwan).

318. 103. EXPLORING THE ANTIPROLIFERATIVE POTENTIAL OF THE ETHYL ACETATE EXTRACT FROM COLEUS NEOCHILUS

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Coleus species have diverse ethnobotanical uses, most of them related to their medicinal properties. Previously, we described the antiproliferative effect of the ethanolic extract of *Coleus neochilus* (also known as boldo rastrero). This study focused on the effect of one of the subfractions of the ethanolic extract, the ethyl acetate fraction (BRAE), on human breast cancer cells. Four different fractions were obtained from the original ethanolic extract by using different solvents: hexane, chloroform, ethyl acetate and methanol-water. Incubation with BRAE 25 µg/ml reduced cell viability in MCF-7, T47-D, and MDA-MB-231 tumor cells (35%, 21% and 8% reduction,

respectively; $p < 0.05$, MTT assays), but not in MCF-10A non-tumor cells. The effect on the reduction of cell viability was also observed when cells were exposed to a 6 or 12 h pulse of BRAE, followed by a 24h-recovery period (14% and 55% reduction respectively, $p < 0.05$), while a 3 h pulse of BRAE did not cause any effect. A concomitant reduction in p-ERK levels measured by western blotting was observed after 6 and 12 h pulses of BRAE. For MCF-7 cells, using a concentration of tamoxifen (Tx) that fails to significantly decrease viability (25 µM) we observed that Tx treatment after 8 h BRAE pulse followed by a 24 h-recovery period was more effective to reduced viability than BRAE alone (BR: 21%, BR+Tx: 66% reduction). For T47-D cells, the treatment of tamoxifen 25 µM (Tx) after a 8 h BRAE pulse followed by a 24 h-recovery period was more effective to reduced viability than Tx alone (BR: 22% reduction, Tx: 21% reduction, BR+Tx: 42% reduction). In addition, BRAE 25 µg/ml decreased 81% the cloning efficiency of MCF-7 cells ($p < 0.05$). The antitumor effect of this ethanolic subfraction was stronger than that described previously for the total ethanolic extract. Therefore, this subfraction is a promising starting point for the purification and characterization of new compounds with potential applications in cancer treatment.

319. 111. COMPLEXITY-INCREASING ALGORITHM FOR THE DETECTION OF MOLECULAR ALTERATIONS IN DIFFERENTIATED PEDIATRIC THYROID CARCINOMA

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Differentiated thyroid carcinoma (DTC) is the most common tumor of the endocrine system in children and its incidence is rising. Molecular markers, either chromosome fusions or single nucleotide polymorphisms (SNPs) serve as prognostic and/or specific treatment-selection tools. Our aim was to characterize molecular alterations in a series of pediatric cases with DTC from Argentina and test a future laboratory algorithm for molecular diagnosis and stratification. FISH, IHC and Sanger sequencing were performed on 57 pediatric DTC biopsies enrolled between 2018 and 2022 at our hospital. The classic variant was predominant 25/57 (47%), followed by the Follicular variant 14/57 (25%). Initially, 4/57 (7%) cases were positive for pan-TRK by IHC and subsequently positive for NTRK3 by FISH. In independent FISH reactions, 2 cases were positive for *ETV6* as the fusion partner. The 2 uncertain cases were solved by NGS and also found to be *NTRK3-ETV6* fused. All negative cases were assessed for other fused genes by FISH. Considering all studied gene fusions, 17/57 (30%) harbored fusions in known oncogenes: 6 in *RET* (35%), 5 in *ALK* (29%), 4 *NTRK3* (24%), 1 *BRAF* (6%) and 1 *MET* (6%). *BRAF* c.1799A>T (p.V600E) SNP was detected in 7/57 (12%) cases by Sanger sequencing. When relating clinic-pathological features with molecular markers, tumor size (T1/T2 vs T3/T4) was significantly larger in those DTC with genetic alterations ($P = 0.027$) and, initial risk assessment (high/intermediate vs low risk) was also statistically higher for cases with genetic alterations ($P = 0.018$). The most commonly fused gene was *RET*, followed by *ALK* and *NTRK3*. Gene fusions were more prevalent than V600E SNP. Designing a laboratory algorithm, following an increasing order of complexity, will provide a reliable molecular testing platform to reduce the requirement on NGS service, which is not available in all laboratories. These results also broaden data on DTC alterations in children from our own geographic region.