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NEUTRON CAPTURE THERAPY RESEARCH AT INFN AND UNIVERSITY OF PAVIA

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University of Pavia has a long tradition in research concerning the application of neutron radiation to medicine, in particular in the radiotherapy exploiting neutron capture. After many years of preclinical activity performed *in vitro* and *in vivo* on animal models, at the beginning of 2000, the Boron Neutron Capture Therapy (BNCT) was applied to two patients affected by disseminated hepatic metastases, that were not surgically operable. After an infusion of BPA-Fructose, the livers of the patients were explanted and irradiated for 10 min in the irradiation facility designed on purpose and constructed inside the Thermal Column of the Triga Reactor. Finally, the organs were re-implanted in the patients. Presently, the research is very active at a preclinical level to extend the application of BNCT with collimated neutron beams to other diffuse tumors as limb osteosarcoma, pulmonary tumors and mesothelioma. A strong collaboration has been built between researchers of the National Institute for Nuclear Physics (INFN), various Italian universities and some international institutions and laboratories working in various aspects of BNCT: from the development of more selective new boron carriers, boron concentration measurements in biological samples by alpha spectrometry, to toxicity and effectiveness tests of the new carriers performed both *in vitro* and *in vivo* in a neutron irradiation position with low gamma background.

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STUDIES OF ANTI-CANCER ACTIVITIES OF NATURALLY OCCURRING TERPENE EXTRACTED FROM THE SEEDS OF PITHCELLOBIUM DULCE PLANT

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Solvent extraction of pre-processed dry seeds of regionally available Pithcellobium Dulce plant was executed using a Soxhlet apparatus with the help of various organic solvents such as methanol, ethanol, acetone, ethyl acetate and n-hexane. All crude extracts were analyzed for investigating phytochemicals. Initially chemical tests were carried out on extracts and on the powdered specimen using a standard procedure to identify the constituents as described by Harborne (1), Sofowora (2), Trease and Evans (3), Siddiqui and Ali (4). The tests were performed to detect the presence of the active chemical constituents like tannin, saponin, quinone, phenol, steroid, flavanoid, caradiac glycosides, terpenoid. The obtained results were further confirmed by High resolution liquid chromatography mass spectrometry (HR-LCMS) and Gas chromatography mass spectrometry (GC-MS). The terpene based molecules were reported in n-Hexane, Ethyl acetate, Acetone and Methanol extracts. The obtained extracts were further analyzed to separate terpene based molecules using various chromatographic techniques. All extracts were subjected to thin layer chromatography (TLC) followed by column chromatography (CC). The obtained extract from column chromatography was further subjected to evaporation of solvent and re-crystallization of obtained terpene based molecules. One of them was oleanolic

acid, which is tri-terpen class of molecule, and was used for further analysis. The structure and compound verification were executed by NMR & Mass Spectroscopy. The isolated Oleanolic acid was then tested for anti-cancer activity. MDA-MB-Human adenocarcinoma mammary gland, K.B. – Mouth and Hela-Human cervix cell lines were used for *in vitro* analysis. Oleanolic acid on MDA-MB, KB and Hela cell line caused IC_{50} values of 10 μG , <10 μG and 10 μG respectively. *In silico* studies on the interaction of Oleanolic acid with CDKs were also performed. The reported ΔG for Non-GA docking and GA-docking were -11.02 and -14.65 Kcal/mol, respectively. The analysis also reported a higher cluster value of 31, which is in consistence with our previous studies (5). In conclusion, Oleanolic acid seems a good anti-cancer agent as reported from our wet laboratory experiments and supported by an inhibition study using molecular modeling techniques.

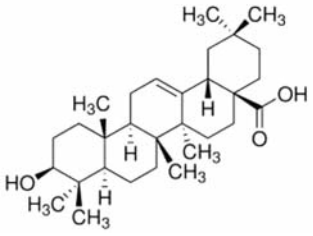

Figure 1. *2D structure of Oleanolic acid.*

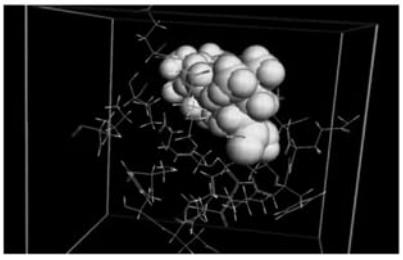

Figure 2. *Docking of Oleanolic acid with 1GII binding site showing inhibition.*

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GOSSYPOL ANALOGS AND BCL-2 PROTEIN – NATURAL COMPOUNDS AND NATURAL TARGET

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Gossypol is a natural phenol derived from the cotton plant, Gossypium species. This compound protects the cotton plant from insects and diseases. Gossypol acts as a BH3 mimetic and interferes with the anti-apoptotic functions of Bcl-2 family proteins. Over-expression of anti-apoptotic proteins is a common feature of cancer cells including chronic lymphocytic leukemia (CLL) and the balance between anti- and pro-apoptotic proteins dictates the fate of neoplastic cells. In addition, microenvironment-mediated signaling further enhances expression of these proteins. Bcl-2 family members are divided into three subgroups based on their function and structural homology. They are anti-apoptotic that includes Bcl-2, Bcl-XL, Bcl-w, Mcl-1, Bcl-B, and BCL2-A1/Bfl-1, pro-apoptotic Bax and Bak and lastly BH3 only proteins such as Bim, Bid, Bad, Noxa and Puma. The major role of anti-apoptotic proteins is to either bind directly with pro-apoptotic proteins to block apoptosis or to bind and sequester pro-apoptotic activators to inhibit Bax and Bak activation. Because anti-apoptotic proteins bind to pro-apoptotic proteins through BH3 domain, efforts have been made to disrupt this interaction resulting in liberation of pro-apoptotic proteins and induction of cell death. This approach provides a rationale to use gossypol as an anticancer agent. Using chronic lymphocytic leukemia as a model system, we elucidated the mechanism of action of gossypol. To take advantage of gossypol's positive attributes while diminishing its negative properties, gossypol analogs such as AT-101, apogossypol and its analogs, gossypolone, and apogossypolone have been synthesized. Success of these compounds and this strategy resulted in an increased interest in synthesizing small molecule inhibitors that target limited – or one member of the Bcl-2 family – anti-apoptotic proteins. This was the genesis of ABT-737 that targets Bcl-2 and Bcl-XL and then ABT-199 that targets only Bcl-2. Our *in vitro* investigations with primary human leukemia cells elucidate the mechanism of action of these agents and their use as single agents or in mechanism-based combinations.

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WNT SIGNALING AS A TARGET OF ANTI-BREAST CANCER DRUG DISCOVERY

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The canonical Wnt signaling pathway plays critical roles in embryonic developmentbut is also extremely important for adult life of all multicellular organisms, including humans. The pathway controls cell proliferation and differentiation, and thus is necessary for the support of stem cell proliferation and self-renewal as well as for regenerative processes. On the other hand, aberrant activation of the Wnt pathway in an adult tissue can lead to carcinogenesis, most notably in the colonand breast.Although the triple-negative breast cancer (TNBC) constitutes about 15% of all breast cancers, its death rate is disproportionally high, accounting for more than 50% of breast cancer deaths. TNBC is highly aggressive and exhibits poor prognosis, being insensitive to the conventional targeted anti-breast cancer treatments due to absence of expression of the receptor tyrosine kinase HER2 and the estrogen receptor. Thus, development of novel therapies is imperative to combat TNBC. One of the approaches in this direction is the development of drugs specifically targeting the Wnt/FZD signaling pathway, critically up-regulated in TNBC cells. One approach in this direction involves development of human antibodies against upstream extracellular components of the pathway. Screening of libraries of synthetic small molecules is another approach to derive Wnt signaling inhibitors. Rational drug design and in silico screening is also applicable to the Wnt pathway. Finally, natural products such as medicinal plant extracts can become a source of novel targeted anti-Wnt therapies. This talk will aim at a general overview of the Wnt/FZD signaling pathways, their implication in breast cancer, and the ways to target this type of signaling in attempts to develop anti-cancer therapies. Special attention will be given to research conducting in our lab.

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TARGET PI3K/AKT-RELATED LIPID KINASE PATHWAYS FOR TREATMENT OF ADVANCED CANCER

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Phosphatidylinositol-4-phosphate 5-kinase alpha (PIP5Kα) is a lipid kinase that acts upstream of phosphatidylinositol 3-kinase (PI3K). Little is known about the role of PIP5K1α in cancer progression. We report that overexpression of PIP5K1α correlates with elevated levels of AKT2, androgen receptor (AR), and CDK1 in primary and metastatic prostate cancer. Elevated levels of PIP5K1α have been associated with poor prognosis of prostate cancer. Overexpression of PIP5K1α increased AKT activity and survival/invasive malignant phenotype in non-malignant prostate cells, while siRNA-mediated knockdown of PIP5K1α in aggressive PC-3 cells led to a reduced AKT activity and down-regulation of cell cycle proteins. We further discovered and developed a small molecule inhibitor that selectively inhibits PIP5K1α in cancer cells. PIP5K1α inhibitor significantly inhibited growth of tumor cells in xenograft mice. We show that the tumor inhibition effect of PIP5K1α inhibitor is mediated by targeting PIP5K1α-associated PI3K/AKT and downstream survival, proliferation and invasion pathways. Thus, PIP5K1α and its small molecule inhibitor have a high potential as drug target and compound for further development of targeted cancer therapy.

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EPIDEMIOLOGY, SCREENING AND VACCINATION OF HPV AND CERVICAL CANCER IN CHINA

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Cancer represents over 25% of total deaths in China. Even with China's socio-economic progress, cancer risk factors (such as smoking) are still not well-controlled. Especially, the infectious agents accounted for 29.4% of cancer deaths in 2005 (31.7% in men and 25.3% in women) in China, compared with 7.4% of cancers deaths in developed countries. Cancer victims return to poverty even if their life had previously been improved. It creates enormous challenges in health care. It has been well known, that early detection and prevention are the major strategies to reduce

cancer mortality by approximately one third if extant technical methods are properly used. However, less than 10% of the cancer patients in China are early cases when first seeking medical advice. The prevention priorities of organ-specific cancer are lung, liver, stomach, esophagus, colon/rectum, breast, cervix and nasopharynx. Started from 2004, 15 cancer control demonstration projects have been initiated in different health infrastructure and economic resources settings. After over 10 years of comprehensive screening studies and evaluating the success of demo projects, China has now put the cervical and breast cancer screening of rural women between 35 years and 59 years firmly on ambitious health reform plan. Between 2009 and 2012, over 20 million rural women were able to access free screening for cervix, while 2.4 million women for breast cancer. This is a step towards provision of universal prevention for screenable cancers nationwide. The public health challenge is substantial. This historical step needs to be followed by developing more appropriate health-care policies to achieve access to health-care services for all Chinese citizens for all preventable cancers.

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E-CADHERIN -160 C/A GENETIC POLYMORPHISM AND CERVICAL INTRAEPITHELIAL NEOPLASIA

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Cervical cancer has been associated with altered expression of E-cadherin. E-cadherin genetic polymorphisms have as a consequence the synthesis of a modified amount of E-cadherin. The purpose of the present study was the analysis of the relationship between tumor necrosis E-cadherin -160 C/A genetic polymorphism and cervical intraepithelial neoplasia. A pilot prospective case-control study was performed, enrolling 37 cases of cervical intraepithelial neoplasia and 50 controls (women with normal cytology and colposcopy). Every patient had a complete gynecological examination with cervical sampling and colposcopy and E-cadherin -160 genotyping. The presence of CA genotype is linked to a high risk for high grade intraepithelial lesion (OR=3.2667, CI 95% [0.8927, 11.954], p=0.0327). The present data encourage the use of E-cadherin genotyping for the management of intraepithelial cervical lesions even though further studies are necessary.

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