PROGRAM MEACR 2012

1st day: Tuesday 27 November, 2012-11-19

8:00-10:00: Registration
10:00-10:30: Opening ceremony
10:30-11:00: Coffee break

Session 1: CANCER GENETICS

Chairpersons: Ala-Eddin Al Moustafa & Sébastien Peffer

11:00-11:40 Plenary Conference 1: Diffuse large B cell lymphoma in Pakistan: Molecular genetics, Prognostic and Predictive implications, Shahid Pervez (Pakistan)

11:40-11:55 OC1: Impact of TGFβ on miRNA expression in Epstein-Barr virus (EBV) infected cell lines, Ali Fendri (Tunisia)

11:55-12:10 OC2: P53 gene mutations in Tunisian patients with chronic lymphocytic leukemia: molecular investigations and correlation with clinical parameters, Ichraf Rezgui (Tunisia)

12:10-12:20 OC3: BRCA1 gene status in breast cancer patients from Kuwait: the involvement of D693N and E1038G variants, Moussa Alkhalaf (Kuwait)

12:20-12:35 OC4: XRCC1, ERCC2 and ERCC3 genes polymorphisms, smoking and occupational exposure and the risk of head and neck cancer in Tunisian population, Rim Khlifi-Slama (Tunisia)

12:35-12:50 OC5: Illumina’s Next-Generation Sequencing Technology at the Service of Cancer Research, Mohamed Chaïr (AGBL AFRIQUE)

12:50-14:30 Lunch

Session 2: CANCER GENETICS

Chairpersons: Shahid Pervez & Ali Gargouri

14:30-15:10 Plenary Conference 2: High-Risk Human Papillomaviruses: Carcinogenesis and Prevention Strategies, Ala-eddin Al Moustafa (Canada)

15:10-15:25 OC6: Mutation screening of RET proto-oncogene in a Moroccan patient with medullary thyroid carcinoma, marfanoid habitus and pheochromocytoma; from clinically MEN2B to genetically MEN2A syndrome, Latifa Hilal (Morocco)

15:25-15:40 OC7: FOXE1 5’UTR variant (rs1867277), which is associated with papillary thyroid carcinoma, could play a role in Graves’ disease genetic susceptibility, Rihab Kallel (Tunisia)
15:40-15:55 OC8: Age distribution of breast cancer in Khorasan Razavi Cancer Registry, Northeast of Iran, Homaei Shandiz (Iran)

15:55-16:25 Coffee break and poster visit

**Chairpersons:** Kailash C. Chadha & Sami Aifa

16:25-17:10 Plenary Conference 3: Host-virus interactions and microRNAs in mammals, Sébastien Peffer (France)

17:10-17:25 OC9: Overexpression of microRNA-10b in nasopharyngeal carcinoma biopsies from Tunisian patients, Nesrine Allaya (Tunisia)

17:25-17:40 OC10: Is It Safe to Leave the Head Open, SAFI UR REHMAN (Ethiopia)

2nd day: Wednesday 28 November, 2012-11-19

**Session 3:** CANCER BIOMARKERS

**Chairpersons:** Abderraouf Kenani & Ala-eddin Al Moustafa

9:00- 9:40 Conference Plenary 4: New Serological Biomarkers for Early Diagnosis and Management of Prostate Cancer, Kailash C. Chadha (USA)

9:40- 9:55 OC11: Tissue Proteomics of Ovarian Cancer, Mohamed EL AYED (Tunisia)

9:55- 10: OC12: IGFR, IL-6R and Akt malignant plasma cell genes expression enhance after Dexamethasone-Thalidomide Therapy, Ines Safra (Tunisia)

10:10-10: OC13: Distribution of Epstein-Barr viral load and anti-EBV antibodies concentrations in serum of individuals from nasopharyngeal carcinoma high-risk families in Tunisia, Nehla Mokni Baizig (Tunisia)

10:25-11:00: Coffee break and poster visit

**Chairpersons:** Seyed Al Moallem & Raja Gargouri

11:00-11:40 Conference Plenary 5: Kidney Cancer Epigenetic Majed S. Alokail (Saudi Arabia)

11:40-11:55 OC14: Frequent CpG methylation of ubiquitin carboxyl-terminal hydrolase 1 (UCHL1) in sporadic and hereditary Tunisian breast cancer patients: clinical Significance, Fatma Trifa (Tunisia)

11:55-12:10 OC15: Descriptive analysis of molecular subtypes in Tunisian Breast cancer Asma Fourati (Tunisia)

12:10-12:25 OC16: Low-level arsenic exposure is associated with bladder cancer risk and cigarette smoking: A case-control study in Tunisian men, Molka Feki-Tounsi (Tunisia)

12:25-14:30 Lunch
Session 4: CANCER THERAPY

Chairpersons: Majed S. Alokail & Ahmed Rebai

14:30-15:10 Plenary Conference 6: Synthesis and potent MDR reversibility of 10-imidazolylacridinediones in breast cancer cell lines Seyed Al Moallem (Iran)

15:10-15:25 OC17: New medicinal plants as promising antitumor drugs
Seyed Hadi Mousavi (Iran)

15:25-15:40 OC18: Ethyl acetate fraction of pansy (Viola tricolor) induces cytotoxic effect against N2a cancer cell line, Ahmad Ghorbani (Iran)

15:40-15:55 OC19: Anti tumor activity of some known drugs on breast cancer, Jihene Elloumi (Tunisia)

15:55-16:10 OC 20: Inhibition of growth and induction of apoptosis In lovo colon cancer cell lines by thymoquinone, Hanene Jrah Harzallah (Tunisia)

16:10-16:40 Coffee break and poster visit

Chairpersons: Hilal latifa & Seyed Hadi Mousavi

16:40-17:20 Plenary Conference 7: Association of Anti- Histamine Drugs with Brain Tumor, Samreen Feroz (Pakistan)

17:20-17:35 OC22: Osteoclast cytomorphometry and activity are mediated by α-tocopherol acetate in Walker 256/B tumor osteolytic rats, Riadh BADRAOUI (Tunisia)

17:35-17:50 OC23: Pathological Evaluation of Breast carcinoma specimen undergoing neoadjuvant chemothérapy Imen TANVIR (Pakistan)

18:00-18:20 Closing ceremony

18:30-19:00: Buffet

3rd day: Thursday 29 November, 2012-11-19

9:00- 10:00: Meeting of the MEACR executive committee members
10:00 Excursion to Kairouan city and its historic mosque (places limited, priority to non-Tunisian participants)
PLENARY CONFERENCES
Diffuse large B cell lymphoma in Pakistan: Molecular genetics, Prognostic and Predictive implications

**Shahid Pervez.**

Professor & Consultant Histopathology, Department of Pathology and Microbiology, The Aga Khan University Medical Centre, Karachi, Pakistan.

Diffuse large B-cell Lymphoma (DLBCL) is extremely prevalent in Pakistan and constitutes about 75% of all B-NHLS in Pakistani adults. Current Literature suggests DLBCL to be a heterogeneous disease which is treated homogenously with widely variable outcome. *Bcl2*, *Bcl6* and *p53* genes seem to have particular relevance in this regard and their alterations may suggest different origin, biological behavior, treatment response and overall survival. In our study a total of 117 adult well-characterized DLBCL were included. The panel of antibodies comprised *Bcl2*, *Bcl6* and *p53* while *PCR* was employed to correlate the events at DNA level in *Bcl2*. The frequency of *Bcl2*, *Bcl6* and *p53* immunohistochemical expression (IHC) was observed in 64.10%, 37.60% and 52.13% cases respectively. Amplifiable quality DNA was available from 90 cases. *Bcl2/IgH* translocation was found in 35/90 patients (38.88%) with 24 cases showing *Bcl2/IgH* (MBR) and 11 cases *Bcl2/IgH* (mcr) translocation. Clinical data was available for 52 patients treated by CHOP therapy. It was found that patients with p53 overexpression had decreased overall survival (*P=0.0004*) whereas *Bcl2* & *Bcl6* expression and *Bcl2/IgH* translocation had no statistically significant impact on overall survival. Our data suggests that simple p53 expression by IHC at the time of diagnosis may help to identify high risk patients who may benefit from more aggressive and newer treatments.
Human Papillomaviruses in the Syrian Population: Carcinogenesis and Prevention Strategies

Amber Yasmeen¹,², Andrew Darnel¹,², Amal Kassab¹, Lina Ghabreau³, Amal Achkhar³ Nizar Akil³ & Alaa Eddin Al Moustafa²,⁴,⁵

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Human papillomaviruses (HPVs) are a group of host-specific DNA viruses, with more than 120 different types identified to date. HPVs are classified as high or low risk (HR or LR) depending on their potential to provoke cancer. It has been demonstrated that HR-HPVs present and play a major risk factor in the development of a variety of human cancers including cervical, colorectal, breast as well as head and neck cancers in the world. However, the presence of HR-HPVs has not been explored yet in the Syrian population. Therefore, we investigated the presence of these viruses and their association with several oncogenes such as Id-1, 2, 3 and 4, as well as P-cadherin and Fascin in human cervical, colorectal, breast as well as head and neck cancers in the Syrian population. Our data showed that high-risk HPVs are present in 96%, 54%, 61% and 43% of these cancers, respectively. Moreover, we noted that the presence of E6/E7 onco-proteins of HR-HPVs is associated with cancer invasiveness in the majority of cancer cases; this is accompanied by an over-expression of Ids, P-cadherin and Fascin genes which are important regulators of cell invasion and metastasis. Thus, in my presentation, I will talk about the presence of HR-HPVs in human cervical, colorectal, breast as well as head and neck cancers in the Syrian population and the usefulness of the available vaccines in this population.
Host-virus interactions and microRNAs in mammals

Sébastien Pfeffer
Architecture et Réactivité de l’ARN, Université de Strasbourg, Institut de Biologie Moléculaire et Cellulaire du CNRS, Strasbourg, France

Micro (mi)RNAs are small regulatory RNAs found in almost all eukaryotes. As they play key roles in fine-tuning fundamental biological processes such as anti-viral defense or cell cycle control, it is quite logical that viruses have evolved to counteract or utilize these molecules. More interestingly, some viruses, especially DNA viruses from the herpesvirus family, encode their own set of miRNAs. They can act as regulators of both host and viral genes expression, ensuring an optimal environment for the virus. Thus, miRNAs, both of cellular and viral origin, are at the very heart of host-pathogen interactions. I will discuss the impact of viral infection on host miRNA expression, and present some evidence that viruses can sometimes counteract deleterious effects mediated by cellular miRNAs. I will also present an overview of virally encoded miRNAs and their expression during infection, followed by examples of some biological roles of these viral regulators. Although we are only starting to fully grasp the importance of viral miRNAs, it is evident that evolutionary distant viruses have evolved to express miRNAs towards convergent functions. Finally, we will also see that getting a precise insight into the relative importance of cellular and viral miRNAs during infection is much harder than previously thought.
New Serological Biomarkers for Early Diagnosis and Management of Prostate Cancer

Kailash C. Chadha,

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Prostate cancer (CaP) is the most common form of non-cutaneous malignancy among men in the United States and the second leading cause of male cancer mortality. CaP mortality rate in African-American men are more than twice as high as rates in white men. Therefore, the key to success in the management of CaP lies in selective and specific diagnostic/prognostic biomarkers. Serum level of prostate-specific antigen (PSA) is at present considered to be the best tumor marker for CaP detection and management. However, the value and the appropriate use of PSA screening remain controversial. There are many drawbacks to this test: a) PSA cannot always accurately differentiate between benign prostatic hyperplasia (BPH) and CaP, especially when PSA levels are between 2-20 ng/ml; b) PSA measurements alone are not able to distinguish between clinically important CaP from indolent-carcinoma; c) its inability to predict metastatic potential and therefore, patient prognosis. Identification of additional serological biomarkers that will either alone or in combination with PSA test will facilitate an early detection/management of prostate cancer and in distinguishing between indolent versus aggressive form of cancer are urgently needed. Aim of this study is to identify a biomarker or a panel of biomarkers that is more sensitive and more specific than currently available PSA test in differentiating BPH from clinically relevant CaP. Progression of CaP is accompanied by modulation in the expression of several key regulatory molecules including VEGF, IL-8, IL-6, bFGF, TGF-β, uPA, TNF-α and IGF-1 etc., and their receptors. Based upon our studies and available evidence in literature, we have evaluated the relevance of differences in levels of several cytokines for the purpose of early diagnosis and management of CaP. Our hypothesis is that CaP patients have in their circulation altered levels of growth regulators such as IL-8, TNF-α and sTNF-R1 that are involved in some aspects of tumor growth & metastasis. The monitoring of the changes in the levels of these regulatory molecules (PSA, TNF-α, sTNFR1, and IL-8) in the serum provides us with new tools that are be more selective and specific in early diagnosis and management of this lethal disease.
Kidney Cancer Epigenetic

Majed S. Alokail

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Epigenetics is the heritable changes in gene expression that are, unlike mutations, not attributable to alterations in the sequence of DNA. It is vital that research is conducted to better understand the epigenetic mechanisms in human cancers, as well as to identify genetic and epigenetic variations that can differentiate between the different subsets of cancers. Our study focused mainly on identifying differential methylation that can distinguish between conventional clear cell renal cell carcinoma (ccRCC), papillary RCC, chromophobe and oncocytoma. By employing a candidate gene approach investigating the methylation profile of known genes associated with cancers such as the RASSF family and genes involved in the hippo pathway. We analysed genome wide methylation study using the Illumina Infinium HumanMethylation 450 bead array, to identify differentially methylated loci. Preliminary results have providing an insight help to identify innovative therapeutic targets.
Synthesis and potent MDR reversibility of 10-imidazolylacridinediones in breast cancer cell lines

Seyed Adel Moallem¹,²,³, Farzin Hadizadeh⁴, Niloufar Dehghani¹
¹School of Pharmacy, ²Pharmaceutical Sciences Research Center, ³Medical Toxicology Research Center, ⁴Biotechnology Research center; Mashhad University of Medical Sciences, Mashhad, Iran

Breast cancer is the most common cancer in women worldwide and is also the leading cause of death from cancer among women globally. Nowadays multidrug-resistant (MDR) breast cancer cell strains have become a major problem in treating breast cancer. With these cells that have reduced susceptibility to routine chemotherapeutic agents, there is clearly a need for a pipeline of novel anti cancer drugs to combat the MDR strains. In this study, four novel 10-imidazolyl acridine 1,9diones derivatives (3a-d) have been synthesized by the reaction of 5,5-dimethyl-1,3-cyclohexanedione (1) with aromatic aldehydes (2a-d) in the presence of ammonia in methanol. In vitro cytotoxicity assessment of the synthesized 10 imidazolylacridinediones derivatives in combination with doxorubicin on T47D and TAMR-6 (tamoxifen-resistant T47D) breast cancer cell lines was investigated using MTT test. Flow cytometry experiments were also implemented to distinguish cells undergoing apoptosis from those undergoing necrosis. Obtained results from MTT and flowcytometry experiments indicated that the 3c derivative (cyanide substitution) along with doxorubicin significantly increase the doxorubicin cytotoxicity in T47D and TAMR-6 cell lines. Meanwhile, the 3d compound (nitro substitution) with doxorubicin did not exhibit a significant synergistic effect on cytotoxic activity of doxorubicin as the 1 nM concentrations and above (100, 10000 nM) of this derivative could slightly increase doxorubicin cytotoxicity in T47D and TAMR-6 cell lines. The synthesized 3b compound (thioethylsubstitution) in concentrations 0.1 nM and above (1, 100, 10000 nM) could potentiate the cytotoxicity of doxorubicin on T47D and TAMR-6 cell lines. On the other hand, 0.1, 1, 100 and 10000 nM concentrations of the 3a derivative (thiomethylsubstitution) exhibited synergistic effect on the cytotoxic activity of doxorubicin in T47D and TAMR-6 cell lines. The most effective synthesized imidazolylacridinediones derivatives was 3c which exhibited rational synergistic effect in combination with doxorubicin in both normal and tamoxifen resistant breast cancer cell lines. The study of best formulation of this compound in combination with doxorubicin is interesting in this regard and is underway in our laboratories.
Association of Anti-Histamine Drugs with Brain Tumor

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Memon Cooperative Housing Society, Karachi - Pakistan

The purpose of this project is to find the association of antihistamine drugs with developing brain tumor and to prove whether using antihistamine drugs increases the risk of developing brain tumor. The link between the antihistamine drugs and brain tumor can be proved by studying the biochemical pathways.

Brain tumor is basically an abnormal mass of tissue in brain in which some cells grow and multiply uncontrollably, however no known cause of brain tumor has been established.

Antihistamine drugs are the type of synthetic drug which selectively counteract the pharmacological effects of histamine. Histamine has major role in different brain functions.

We started our research by studying different antihistamine drugs; their mechanism of action and respective target sites, performed sequence similarity database searching and performed alignment of multiple related sequences. Further, we identified the functional sites of drug’s receptor proteins.

In the final stages of the project we performed reverse docking to target down the repetitive proteins that cause a detrimental enhancement in the brain tumor and are involved in its overall growth. The proteins were then docked with the cimetidine drug to find out the close proximity and binding status of the drug with the protein. This binding will direct us towards the better locked protein for which cimetidine will act as an active tumor inhibitor.
ORAL PRESENTATION
Impact of TGFβ on miRNA expression in Epstein-Barr virus (EBV) infected cell lines

Ali Fendri¹, Lassad Oussaief², Béatrice Chane-Woon-Ming¹, Irène Joab² and Sébastien Pfeffer¹.

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MicroRNAs (miRNAs) are a large class of small (22nt) non-coding RNAs that negatively regulate gene expression, and have been shown to be key regulators in a variety of processes including development, cell cycle and immunity. Epstein-Barr virus (EBV) is an oncogenic gammaherpesvirus, endemic in humans, that encodes for twenty-five miRNAs. It is associated with several well-recognized malignancies, such as Burkitt’s lymphoma, and nasopharyngeal carcinoma.

We performed small RNA deep-sequencing from the EBV-positive Burkitt’s lymphoma cell lines Mutu-I, Sav-I and Kem-I, following both 2h and 24h of TGFβ treatment to identify differentially expressed miRNAs. A markedly differential expression of cellular and viral miRNAs was observed between on one hand the Mutu and Kem cell lines and the Sav cell line on the other hand. This difference is most likely due to the expression of the oncogenic protein LMP1 by the Sav cell line. Interestingly, induction of the lytic cycle via TGFβ or the expression of LMP1 could be responsible for the up-regulated expression levels of some EBV miRNA: BHRF1-2 and BART7 and the oncogenic cellular miRNAs mir-155 and mir-146a. These observations were confirmed by real time RT-PCR and northern blot analysis in all cell lines tested. In addition, we also noticed a dramatic difference in the absolute amount of these two cellular miRNAs between the three cell lines analyzed. The regulation of these miRNAs via LMP1 may thus represent a key event in the lymphomagenesis of EBV positive Burkitt’s lymphoma.
Session 1: CANCER GENETICS

Oral presentation: 2

P53 gene mutations in Tunisian patients with chronic lymphocytic leukemia: “molecular investigations and correlation with clinical parameters”

Ichraf Rezgui, Ines Safra, Chaker Fouzai, Slah Ouarhani and Salem Abbes

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Chronic lymphocytic leukemia (CLL) is defined by the accumulation of mature and monoclonal B lymphocytes in the blood and bone marrow. This pathology is associated with various genetic alterations involving oncogene and tumor suppressor genes. The P53 is one of the most studied tumor suppressor genes associated with CLL. The gene is located at 17p13.1 and contains 11 exons. Moreover, it was demonstrated that this gene is mutated in approximately 50% of human cancers. In this proposal, we intend to investigate P53 mutations in Tunisian patients affected by CLL.

60 adult patients referred to our laboratory were investigated for P53 mutations at exons 5, 6, 8 and 9 known to be hot spot mutation regions. DNA was extracted from white blood cells by standard methods using phenol chloform procedure. Mutations were characterized by PCR / sequencing. Statistical analysis was used to correlate the mutational spectrum with clinical parameters of the CLL.

A total of four mutations were detected in our sample, all of them were identified at the heterozygous state. Two accrued at exonic and two at intronic positions giving a total frequency of 8.33%. The exonic mutations were G> T at codon 216 in exon 6 (V216L) and C> G at codon 312 in exon 9 (T312S). The intronic variations were G> C in the acceptor site of exon 9 (IVS8 +92) and C> T at the intron 9 (IVS9 12).

Statistical analysis using the logistic regression test showed a significant correlation between P53 mutation status and score Matutes (p <0.05) but does not show a correlation between P53 mutation status and disease severity (p> 0.05). The follow of this study on a larger patient recruitment added to computing modelization and expression experiment might give a clear clue to the interpretation of the role of such mutation in the occurring of CLL Pathology.
BRCA1 gene status in breast cancer patients from Kuwait: the involvement of D693N and E1038G variants

Moussa Alkhalaf
Department of Biochemistry, Faculty of Medicine, Kuwait University, Safat 13110, Kuwait

The status of BRCA1 gene to breast cancer in Arab women has not yet been explored. The aim of this study was to analyze the spectrum of BRCA1 mutations in breast cancer patients from Kuwait and to identify possible Kuwaiti founder mutations.

We analyzed the entire coding regions of the BRCA1 gene for 106 unselected Kuwaiti breast cancer patients and 114 healthy controls. A family history was obtained from each patient and blood sample was processed for DNA analysis. We used denaturing high-performance liquid chromatography (DHLPC) to screen all exons for possible variants. DHPLC variants were confirmed by direct sequencing.

The frequency of D693N, P871L, E1038G, M1083V and S1040N mutations in healthy controls was 7%, 32.45%, 34.2%, 0% and 0.87%, respectively. The prevalence of these five mutations in breast cancer patients was 18.9%, 24.5%, 41.5%, 0.94% and 2.83% . The mean age of onset in patients with E1038G, D693N and combined mutation D693N and E1038G was 53.4, 47.0 and 46.2 respectively (p<0.05). The frequency of the 2201 C>T polymorphism was 55.6% CC, 38.3% CT and 6.1% TT in controls and 46.3 CC, 43.5% CT and 10.2% TT in cases. The frequency of the 2430 T>C polymorphism was 53.6% TT, 39.3% TC and 7.1% CC in controls and 46.1% TT, 41.2% TC and 12.7% CC in cases.

Our data shows for the first time the spectrum of BRCA1 mutations in breast cancer patients from an Arab population. We show here that the spectrum of BRCA1 mutations in Kuwait is different from all studied populations. We observed that Kuwaiti women with combined D693N and E1038G variants have increased risk for developing breast cancer (p<0.05).

Acknowledgement: Kuwait University Grant # MB04/07
DNA repair enzymes are important to maintain equilibrium of DNA integrity, replication and stability. Altered activity of these enzymes may be involved in modulating cancer susceptibility and pathogenesis of head and neck cancer (HNC). We performed a case-control study to test the association between three common single nucleotide polymorphisms of XRCC1, ERCC2 and ERCC3 genes with HNC risk in Tunisian patients.

The genotype analysis of XRCC1 Arg399Gln, ERCC2 Lys751Gln and ERCC3 A>G polymorphisms for 169 HNC patients and 241 controls were performed using the PCR-RFLP, with the endonucleases MspI, MboII and NcoI, respectively. Stratification of the populations according to smoking habits and occupational exposure highlighted the importance of tobacco and toxic substance as two risk cofactors for the development of HNC (OR = 134.1; P< 0.001 for combined effect of high occupational exposure and heavy smoker). Our study suggests that only the XRCC1 Gln399Arg polymorphism was associated with the risk of HNC in a Tunisian population (OR = 2.04; P = 0.001). Furthermore, we found that the risk of HNC was associated with XRCC1 Gln399Arg polymorphism jointly to occupational exposure status (OR = 2.29; P = 0.024). Also, ERCC3 A>G polymorphism appear to be related to HNCs in the heavy smokers group (OR = 2.79; p = 0.041). However, no statistically significant association was observed for the ERCC2 Lys751Gln polymorphism alone or jointly to smoking and occupational exposure status with the risk of developing HNC.

These data suggest that the Gln399Arg is associated with an increased risk of developing HNC, since it correlates with occupational exposure in Tunisian population.
Illumina’s Next-Generation Sequencing Technology at the Service of Cancer Research

Mohamed Chaïr

Product Specialist Life Sciences/AGBL AFRIQUE

As a leading provider of integrated solutions that advance the understanding of genetics and health, Illumina is working to develop better, more sensitive, cutting-edge cancer diagnostic tools. Our ground-breaking next-generation sequencing technology is transforming genetic research, enabling scientists to peer into the human genome, epigenome and transcriptome at a depth and resolution previously not achievable. With such a wealth of genetic information, researchers are able to compare data across multiple individuals and populations to identify genetic disease associations. As the technology advances, it’s becoming faster, cheaper and more accessible, enabling discoveries that can one day translate into powerful cancer diagnostic tools.
Session 2: CANCER GENETICS

Oral presentation: 6

Mutation screening of \textit{RET} proto-oncogene in a Moroccan patient with medullary thyroid carcinoma, marfanoid habitus and pheochromocytoma; from clinically MEN2B to genetically MEN2A syndrome*

El Annas Abdessamad\textsuperscript{1, 2}, Iraqi Hind\textsuperscript{3}, Fritez Nabila\textsuperscript{1, 3}, Chraïbi Abdelmjid\textsuperscript{3}, Hilal latifa\textsuperscript{2}

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Multiple endocrine neoplasia type 2 (MEN2) is an autosomal dominant inherited multiglandular syndrome and consecutive malignant neoplasia. It is sub-classified into three variants: MEN2A associates medullary thyroid carcinoma (MTC), pheochromocytoma and parathyroid hyperplasia; MEN2B associates MTC, pheochromocytoma, oral, ocular, and alimentary submucosal, intestinal ganglioneuromas, and marfanoid body features; and familial MTC occurs in families in the absence of other manifestation of MEN2.

Germline mutations of \textit{RET} proto-oncogene account for approximately 25\% of all MTC cases and occur as multiple endocrine neoplasia type 2 (MEN2) syndromes. Pheochromocytoma in MEN 2A is associated most frequently with mutations in codon 634 (in exon 11). Subjects with these mutations have a more than 90, 50, and 30\% probability of developing medullary thyroid cancer, pheochromocytoma, and hyperparathyroidism, respectively. MEN 2B is associated with mutations primarily in codon 918 (in exon 16) of the \textit{RET} proto-oncogene. Differentiation between MEN2A and MEN2B is critical for management of such patients, since, in MEN2A, prophylactic thyroidectomy is recommended to be performed in carriers later.

Here we report a patient with marfanoid habitus, MTC, and bilateral pheochromocytoma which is clinically supposed to be matched with MEN2B and finally diagnosed as MEN2A according to genetic tests. Regarding these findings, marfanoid habitus alone is not a strong clinical finding for categorization of MEN2 sub-types and timing of surgery should be recommended according to genetic evaluation and specific codon.

* This study has been supported by the PROTARS/P12-20 project and UATRS-CNRST Morocco
Session 2: CANCER GENETICS

**Oral presentation: 7**

**FOX E1 5'UTR variant (rs1867277), which is associated with papillary thyroid carcinoma, could play a role in Graves’ disease genetic susceptibility.**

**Kallel Rihab, Belguith-Maalej S, Mnif Mouna, Abid Mohamed, Hassen Hadj Kacem**

Centre de Biotechnologie de Sfax, Laboratoire de Microorganismes et de Biomolécules, équipe : Procédés de Ciblage Moléculaires Cellulaire, Sfax, Tunisia.

*FOX E1* is a candidate gene for thyroid diseases. Recently, we have reported the involvement of polyalanine tract (poly-Ala) in the genetic susceptibility of papillary thyroid carcinoma; however no significant association was found with the autoimmune thyroid diseases (AITD). Moreover, our functional analysis suggested that both the SNP (rs1867277:-238G>A) within the *FOX E1* 5'UTR and poly-Ala variations affect *FOX E1* expression in exposed PTC patients. The aim of our study was to assess the association between rs1867277 and the risk of AITD and finally to evaluate the eventual effect of these variants in RNA modeling.

Tow-seventy-seven Tunisian patients affected with AITD (Grave’s disease (GD) (n=129), Hashimoto Thyroiditis (HT) (n=76) and primary iodipathic myxidoma (PIM) (n=72)) and 169 matched healthy controls participated in this study. The rs1867277 was studied using gene-specific PCR single specific primer and restriction fragment length polymorphism. The eventual effect of Ala length and rs1867277 alleles on *FOX E1* expression was investigated by RNA modeling tools (RNAfold web server).

The association of the rs1867277 with HT and PIM was rejected (P>0.05). However, a significant association was found with GD ($\chi^2$=6.54, 2df, p=0.03). In addition, the genotypic distributions revealed the predispositional effect of the A/A genotype (OR=2.59; 95%CI: 1.29-5.23; p=0.003). Nevertheless, no protective effect was observed for the homozygous G/G (OR=1.54; 95%CI: 0.93-2.55; p=0.07).

Bioinformatics analysis showed that the GCC repeat and rs1867277 alleles modulate the predicted RNA secondary structure. Complexity and stability of *FOX E1* RNA structure has been raised with GCC length. Moreover, RNA structures folded with G-allele of rs1867277 were more stable than those with A-allele.

In conclusion, our work highlights the positive association of the *FOX E1* 5'UTR variant rs1867277 with GD. Besides, using bioinformatics tools, A-allele would influence *FOX E1* RNA stability.
Age distribution of breast cancer in Khorasan Razavi Cancer Registry, Northeast of Iran

Homaei Shandiz F¹, Janghorban R², Azarkish F³, Sedigh S⁴, Seyed Amir Aldavood⁵, Sayadi M⁶, Babaei A.

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Introduction: In Iranian women the major malignancy is breast cancer and it is the most frequent cause of cancer death in this group.

Aims: The aim of this study was to describe the age distribution of the incidence of breast cancer in female population in Khorasan Razavi province.

Material & Methods: The study subjects were 1345 patients with breast cancer diagnosed/registered in the two major university clinics between 2000 and 2010 in Khorasan Razavi province. Age – Specific Rate for incidence was calculated for the study population. Total female population of the area was obtained from the National Dejure Census in 2006.

Results: The findings was showed that patients ranged from 20 to 84 years old, with those 40-44 being the most prevalent. Forty-one percent of breast cancer were diagnosed in women younger than 44 years. The mean age of breast cancer presentation was 48.33±11.93. The Age-Specific incidence of breast cancer in Khorasan Razavi was showed an overall increasing trend for age peaking at age 55-59 years, with a declining trend for women older than 59 years.

Conclusion: Age –Specific incidence of breast cancer at younger ages than peaking group was high. Early occurrence in breast cancer is an important issue might persuade health care authorities to plan regional strategies for breast cancer control in female population in Khorasan Razavi province.
Overexpression of microRNA-10b in nasopharyngeal carcinoma biopsies from Tunisian patients

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Micro-ARNs (miRNAs) are effective post-transcriptional regulators of gene expression and are important in many biological processes. Many miRNAs have been reported to function as tumor oncogenes or anti-oncogenes. MicroRNA-10b was identified as an miRNA highly expressed in metastatic breast cancer, promoting cell migration and invasion. Similar findings were also concluded in nasopharyngeal carcinoma (NPC) line, C666-1, harboring the Epstein Barr virus (EBV). In this NPC line, the impact of the viral oncoprotein LMP1 on the miRNA-10b expression was demonstrated via the transcriptional factor Twist-1. The aim of our study was to examine the level of miRNA-10b in tumor specimens of patients with NPC and to investigate its correlation with the expression of LMP-1 and Twist-1. In fact, our analysis by Stem-loop RT-QPCR shows high levels of miRNA-10b expression in NPC samples (n=44) compared to non-neoplastic nasopharyngeal tissues (n=6) (Ratio 11.31; p=0.048). The aberration of miRNA-10b expression was also significantly associated with the large tumor size (T3-T4; Ratio 8; p=0.02), and the juvenile form of noth africain NPCs (patients aged less than 30 years; Ratio 5.65; p=0.028). In contrast, we found no relationships with lymph node or extra-nodal metastases at initial examination. On the other hand, the analysis of LMP1 and Twist1 by RT-PCR showed the significant correlation between simultaneous expression of these genes and the high levels of miR10b, specifically for the undifferentiated histological type (UCNT). Overall, the present data purpose the miR10b as tumor maker of NPC patients and suggest its impact on the NPC proliferation rather than the metastatic process.
Is It Safe to Leave the Head Open

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We present a case of a young girl with a large, fungating, ulcerated forehead tumour with pus discharge and involving both frontal lobes of brain. After radical removal of the tumour, a large dural, skull and scalp defect was treated with fascia lata dural graft only, leaving the scalp open for 5 days. The second procedure consisted of acrylic cranioplasty and skin graft.

Inspired by the dramatic effects of leaving the abdomen temporarily open for abdominal compartment syndrome and even for reducing ICP in TBI, the idea of leaving the head open for a few days may be plausible.

It is well known that decompressive craniectomy reduces ICP in traumatic brain injury and improves brain compliance and cerebral blood flow.

Rare as it is, at times brain may be so swollen that in spite of all medical measures and multiple releasing galeal incisions scalp and skin closure may be difficult. Drastic measures like frontal or temporal lobectomies, removing the seemingly normal brain are the last resorts.

Apart from brain swelling, there may be skin and scalp loss or involved in the inflammatory or neoplastic process, making primary closure impossible.

Skin grafting should be left for definitive closure.

The risks of CSF leak and intracranial infection are real, but as in this case leaving the head open does not invariably lead to disaster.

Details of this dramatic case and favorable outcome will be presented and critical review of the literature discussed.
Tissue Proteomics of Ovarian Cancer

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Direct analysis of tissues by MALDI MS is an interesting strategy for clinical applications (markers hunting of pathologies) by giving direct information at the tumor level. In fact, in a cancerous tissue, most parts of the tissue do present the same tumoral phenotype. Potential markers can be identified and cross-validated using different tools (IHC, western blots, MALDI imaging) to confirm their presence and localization. We have applied this strategy of tissue proteomics for biomarkers hunting of ovarian cancer. Ovarian carcinomas and benign ovaries were directly analyzed by MALDI-TOF-MS. After automatic profiling and mass spectrometry imaging analyses, hierarchical clustering based on principal component analysis in nonsupervised mode was carried out. On the same samples, preparations were performed to investigate peptides, then proteins, followed by high mass proteins, in an automatic profiling to specific signatures for diagnosis. Using tissue bottom-up strategy on tissue digestion, and mass spectrometry imaging after by shotgun sequencing by Nano-LC-IT-MS in MS/MS mode from washing samples from on tissue digested peptides, several biomarkers were found.

Comparison of benign and cancerous biopsies according to the different strategies has allowed obtaining a list of potential biomarkers. Among these biomarkers several were identified. One marker is a fragment of immunoproteasome 11S. This fragment was found by cross validation to be present in epithelial cells. IHC reveals a change in the addressing of the fragment between cancer and benign samples from nucleus to cytoplasm. However, this fragment was not found in epithelial cells of other cancers such as colon cancers but was also found in uterine cancers demonstrating that PA 28 alpha fragment is specific of genital cancers. This underlines the role of immunity failure (immuno tolerance) in cancer cases with invalidation of the immunoproteasome. Interestingly, using MALDI MSI High mass detection strategy coupled to bottom-up procedures, several other biomarkers have been identified. A list of specific biomarkers from the ovarian carcinoma
regions was obtained and classified as proteins associated with cell proliferation, involved in immune response modulation, signaling to the cytoskeleton, and tumor progression. These specific biomarkers were then validated by immunocytochemistry using Tag-mass technology, cell biology, and Western blot, and by PCR (using SKOV-3 ovarian epithelial cancer cells) \(^8\). From several candidate proteins, including profilin-1, cofilin-1, vimentin, and cytokeratin 19 involved in intracellular signaling to the cytoskeleton, some are implicated in the conversion of epithelial cells to mesenchymal cell. This clearly demonstrates the interest of the strategy for finding markers \(^9\). Tissue proteomics has higher potential for markers discovery by searching markers directly at the tumor level and ease their tracking in fluids for diagnosis. Such a strategy allows avoiding pitfalls of markers hunting directly from fluids.
Session 3: CANCER BIOMARKERS

Oral presentation: 12

**IGFR, IL-6R and Akt** malignant plasma cell genes expression enhance after Dexamethasone-Thalidomide Therapy

_Ines Safra^

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**Introduction:** Interleukin-6 (IL-6) and insulin growth factor (IGF) are most important cytokines implicated in malignant plasma cells growth and survival. PI3K/Akt signaling pathway plays a critical role in multiple myeloma pathogenesis and progression. This Study analyses bone marrow plasma cells IL-6R, IGFR and Akt genes expression in multiple myeloma patients at diagnosis and after Dexamethasone-Thalidomide induction therapy, comparing to healthy bone marrow donors. **Patients and Methods:** 47 patients and 16 controls were analyzed, Bone marrow plasma cells were isolated, and genes expression was quantified using Taqman quantitative PCR technology, calculated by 2-\(\Delta\)CT formula, reference gene used was RPLPO. SPSS and STATView logiciels were used to analyze statistics correlations. **Results:** Quantitative IGF receptor (IGFR) gene malignant plasma cells expression at diagnosis was lower than normal ones (p = 0.01), after induction therapy IGFR expression increase significantly (p = 0.04) and reach controls value. Down expression of Akt gene was also observed at diagnosis in 46% of patients (p<0.001), these expression were multiplied by 4.8 after treatment, exceeding significantly controls values (p = 0.003). IL-6R expression was similar in normal and malignant plasma cells at diagnosis, and increases significantly after Dexamethasone-Thalidomide (p = 0.05).

**Conclusion:** In this study, plasma cells IL-6R, IGFR and Akt genes expression was enhanced after Dexamethasone-Thalidomide treatment. This interaction seems to influence treatment response. Clarification of mechanisms implicated in plasma cells IL-6R, IGFR and Akt gene expression control may contribute to best appreciation of pathogenic Multiple myeloma drug resistance ways and discovery of novel specific drugs.
Distribution of Epstein-Barr viral load and anti-EBV antibodies concentrations in serum of individuals from nasopharyngeal carcinoma high-risk families in Tunisia

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Because nasopharyngeal carcinoma (NPC) has a close association with Epstein-Barr virus (EBV), measuring serum EBV DNA and anti-EBV serum marker concentrations could be a feasible method for NPC diagnosis, monitoring and probably screening especially in a community at risk. The aim of this study was to determine the EBV pattern in sporadic NPC and in high risk NPC Tunisian families in order to evaluate their risk factors and help for NPC screening.

The rates of anti-EBV antibodies and EBV DNA were determined in the serum of 47 healthy members randomly selected from 23 NPC multiplex families with two or more affected members, 93 healthy Tunisian community controls chosen with the same age, sex and geographic origin as unaffected individuals and 66 EBV positive sporadic NPC patients whose serum was available before and after treatment.

Unexpectedly, significant lower concentrations of anti-EA (Early Antigen) IgG and anti-VCA (Viral Capsid Antigen) IgG were found in unaffected members from NPC families than in healthy controls while viral loads were negative in all the tested sera. For sporadic NPC patients, anti-EA IgG and anti-VCA IgA concentrations were significantly higher than in healthy controls and these rates decreased after treatment. The level of EBV DNA load varied according to the condition of the tumour.

This study suggests that in the Tunisian NPC families, screening for malignancy is based on serum concentrations but not on EBV DNA load while in the sporadic NPC group, serologic markers and EBV DNA load are complementary for diagnosis and follow-up.
Frequent CpG methylation of ubiquitin carboxyl-terminal hydrolase 1 (UCHL1) in sporadic and hereditary Tunisian breast cancer patients: clinical significance.

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Aberrant methylation of the CpG islands in promoter regions is one of the mechanisms for inactivation of tumor suppressor genes in many human cancers including breast carcinoma. In this study, we aimed to assess, by methylation specific PCR (MSP), the CpG methylation pattern of the UCHL1 promoter in 94 sporadic and 44 hereditary breast cancer from Tunisian patients. The percentage of UCHL1 methylation was 67% in sporadic and 82% in hereditary breast cancer cases. In sporadic cases, UCHL1 methylation correlated with poor response to treatment \((P = 0.042)\) and progesterone receptor status \((P = 0.036)\), whereas in patients with hereditary predisposition, the only significant association was found with Her2 expression \((P = 0.024)\). Moreover, in patients with sporadic breast cancer, the UCHL1 unmethylated pattern conferred a prolonged overall survival time in particular in the group of patients with advanced TNM stage of the disease \((P\text{ log rank} = 0.04)\). Aberrant CpG methylation of the UCHL1 promoter was significantly associated with transcriptional silencing of this tumor suppressor gene in sporadic breast cancer tissues \((P = 0.001)\). On the other hand, the UCHL1 unmethylated pattern correlated with P53 positivity in primary sporadic tumors \((P = 0.032)\), supporting the functional link between the two tumor suppressors in breast tumorogenesis.
Descriptive analysis of molecular subtypes in Tunisian Breast cancer

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\textbf{Aim}: The objective is to report the correlation between pathology and molecular subtypes classifications of breast cancer in Tunisian women.

\textbf{Methods}: This retrospective study concerned data of 966 breast cancer cases collected from 2007 to 2009 at Salah Azaiez Institute of Tunis. These cases were classified by immunohistochemistry test for estrogen and progesterone receptors and human epidermal growth factor receptor 2 status within the four molecular subtypes, namely luminal A, luminal B, human epidermal growth factor receptor 2+ and triple-negative. The molecular classifications were correlated to the clinicopathological characteristics of the tumors.

\textbf{Results}: Luminal A (50.7 \%) was the most common immunohistochemical subtype, while triple negative subtype represented 22.5\% of cases. Both luminal B and human epidermal growth factor receptor 2+ represented 13.4\% of cases. Immunohistochemical subtypes were significantly associated with large tumor size (>5cm, \(p<0.000\)), younger age (<40 years, \(p<0.030\)) and high grade (\(p<0.000\)). Conversely, there was no correlation to the lymph node status.

\textbf{Conclusion}: Our data demonstrated that luminal A subtype, associated with a favourable prognosis, was the most frequent subtype in the Tunisian population, however the triple negative subtype occurred at a high incidence in Tunisia compared to Western countries. The molecular subtypes are correlated to the tumor size, histological grade and patient’s age.
Low-level arsenic exposure is associated with bladder cancer risk and cigarette smoking: A case-control study in Tunisian men

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Although exposure to high levels of arsenic is associated with excess bladder cancer risk, lower exposures generally are not. This study represents a first biomonitoring of arsenic exposure in Tunisia and focus on a possible association with bladder cancer risk. In this context, 1xx male bladder cancer cases and 250 controls were recruited and blood samples were analyzed to determine the concentration of As. The study subjects were stratified into median groups, based on concentrations of arsenic in subjects' blood. Blood arsenic (B-As) was significantly higher (about 2-3 folds) in bladder cancer cases than in controls. The arsenic concentrations were significantly higher among both smokers and workers in construction. However, neither drinking water nor sea food were found to be incriminated as exposure sources. The adjusted risk ratios for B-As concentration categories 0.1-0.67 and Greater/equal to 0.67 microg/L were 1.34 (95% CI, 0.94-1.91) and 2.35 (CI, 1.021-5.40), respectively. The elevated risk in the category of highest cumulative exposure confirms association between bladder cancer risk and low-level arsenic exposure. Further, modulation of arsenic level according to the histological grade may be of potential to be used as diagnostic marker of the disease process and its possible relationship etiologically.
Session 4: CANCER THERAPY

Oral presentation: 17

New medicinal plants as promising antitumor drugs

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Cytotoxic effects of different extracts and obtained fractions from *Salvia chorassanica, Lavandula Angustifolia and Rheum turkestanicum* as Iranian medicinal plants on different cancer cell lines including HeLa, HL60, K562, MCF-7, and lymphocytes as non-malignant cells were studied. Meanwhile the role of apoptosis was explored in this toxicity.

Malignant and non-malignant cells were cultured in RPMI 1640 medium and incubated with different concentrations of plant extracts. Cell viability was quantified by MTT assay. Apoptotic cells were determined using PI staining of DNA fragmentation by flow cytometry (sub-G1 peak). The molecules as apoptotic signal translation, including Bax and cleaved PARP, were identified by Western blot. The degree of DNA fragmentation was analyzed using agarose gel electrophoresis based on the formation of internucleosomal units.

Cytotoxic studies revealed that all compounds exhibited cytotoxic activity against cancer cell lines with IC50 values starting from 7 µg/ml according to type of extract and cell lines. Apoptosis was confirmed as a possible mechanism of these toxicities.

In conclusion *Salvia chorassanica, Lavandula Angustifolia and Rheum turkestanicum* exert cytotoxic effects in different cancer cell lines in which apoptosis plays an important role. IC50 values show these plants could be considered as a potential and promising chemotherapeutic agent in cancer treatment.
**Ethyl acetate fraction of pansy (Viola tricolor) induces cytotoxic effect against N2a cancer cell line**

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**Objective:**  
Recent studies have shown that pansy (Viola tricolor) decreases proliferation of different cell lines. The present study was designed to investigate ethyl acetate fraction of V. tricolor against N2a cancer cell line.

**Material and methods:** The aerial parts of V. tricolor were extracted with 70% ethanol for 48 h using Soxhalet apparatus. Then, the extract was partitioned between H₂O and ethyl acetate. The N2a cells were cultivated and incubated for 24 h with different concentrations (100-800 µg/ml) of the ethyl acetate fractions. The cell viability and expression of markers for apoptosis was determined using MTT colorimetric assay and Western blot analysis, respectively.

**Results:** Following incubation of the cells with 100, 200, 400 and 800 µg/ml of the fraction, approximately 12, 24, 38 and 87% inhibition in cell growth was observed, respectively, as compared with untreated cells. Western blot analysis showed an increase of caspase-3 and ratio of Bax/Bcl2, in the cells treated with 800 µg/ml of ethyl acetate fraction.

**Conclusion:** The results suggest that ethyl acetate fraction of V. tricolor exhibits antitumor activity against N2a cells. Further isolation and purification of the active compound(s) may yield novel anticancer agents.
Antitumor activity of some known drugs on breast cancer

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Breast cancer is the first cause of cancer death in women between 40 and 50 years, it represents a major public health problem.

Breast cancer is clinically classified depending on the expression of estrogen receptor (ER) in a tumor biopsy that could be ER+ or ER−. The ER+ breast tumors represent 50-80% of all breast tumors.

The presence of ER is currently correlated with better survival prognosis and could be treated with the administration of antiestrogens.

The most commonly antiestrogen treatment used is the tamoxifen (over ten million patients per year) but many resistance forms are developed and several side effects remain significant (hot flashes, cataracts, thromboembolic events, endometrial cancer).

The treatment with clomiphene citrate (CC) and estradiol have shown a controversial results in vivo, according to some studies they did not have any effect; according to others demonstrate a cytotoxic or proliferative effect. The purpose of this present study was to evaluate the cytotoxicity of CC and E on breast cancer cell lines MCF7 (ER+) and BT20 (ER−)

Our results show that (CC) has a significant anti-proliferative effect against both MCF7 (p << 0.000001) and BT20 (p << 0.000001) with an IC50 of BT20 higher than the IC50 of MCF7.

The study of the effect of the estradiol (E) has shown an antagonist result; via a kinetic analysis, it appears that E enhances the proliferation of both MCF7 and BT20.

DNA fragmentation test has shown that CC induced apoptosis in both MCF7 and BT20 cells.

We conclude that CC has a cytotoxic effect on either estrogen positive or estrogen negative breast cancer cells however E has a proliferative effect.
**Inhibition of growth and induction of apoptosis In lovo colon cancer cell lines by thymoquinone**

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Thymoquinone (TQ), *Nigella sativa* bioactive compound, has been reported to exhibit anti-oxidant, anti-inflammatory and anti-tumor activities through different mechanisms. However, the effect of TQ on cell signaling and survival pathways in resistant cancer cells has not been fully delineated. This study reported the anticancer effects of TQ on Lovo colon cancer cells, and its potential effect on the EGFR and the phosphotyrosin Kinase activation pathway.

In this approach TQ demonstrated cyto- and genotoxic effects in a concentration dependent manner: it induced significant anti-proliferative effects at 60 µM and acute cytotoxicity at higher concentrations.

TQ treatment decrease cellular levels of EGFR proteins, resulting in a substantial decrease of phosphorylated tyrosin Kinase, a known regulator of cell survival.

**Keywords:** *Nigella sativa*; Thymoquinone; tyrosin kinase; EGFR; lovo cells
Osteoclast cytomorphometry and activity are mediated by α-tocopherol acetate in Walker 256/B tumor osteolytic rats

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Bone is the most common sites of breast cancer metastasis. Metastases often associated bone destruction and are a major cause of morbidity. We examined the effects of vitamin E supplementation (VES) on osteoclast (OC) resorbing activity and cytomorphometry in Walker 256/B tumor osteolytic rats. Twenty-four aged male rats (2 months old) were randomized into 3 groups: 6 were Sham operated; 9 were injected in the right hind limb with Walker 256/B malignant breast cancer cells (W256 group); and 9 were injected as above and supplemented with VE (45 mg/kgBW) (W256VE group). Twenty days after surgery, bones were radiographed and scored. Bone mass (BV/TV) and some microarchitectural parameters (Tb.Th, Tb.Sp, Tb.N, OV/BV, OS/BS) were assessed. Some conventional histodynamic parameters of OC (ES/BS, N.Oc/B.Ar, N.Oc/B.Pm, and Oc.S/BS) were measured. Cellular and nuclear form factors (FF_C and FF_N) were measured for OC populations. The nuclear-cytoplasmic ratio (N/C) was also determined. W256 group exhibited osteolytic lesions in the operated femora. Walker 256/B induced trabecular perforation and decreased BV/TV associated with significant increases in OC numbering (N.Oc/B.Ar and Oc.N/B.Pm) and activity (ES/BS and Oc.S/BS). While FF_N remain unchanged, FF_C and N/C ratio increased in W256 group. W256VE showed less osteolytic lesions. In fact, VES to cancerous rats had alleviative effects on bone loss with cytoinhibition rate reaching 41%. Moreover, disruption of bone microarchitecture and OC activity in W256VE group decreased. VES reduced the malignant Walker 256/B-induced enhanced OC resorbing activity. The protective effect of VE may be due to its modulation of OC cytomorphometry and subsequently their activity.
Pathological Evaluation of Breast Carcinoma Specimens Undergoing Neoadjuvant Chemotherapy

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Purpose: The objectives of study were to assess pathological changes in the breast tumor by using Miller Payne classification in a group of patients undergoing neoadjuvant chemotherapy. Our study aimed to highlight the importance of changing trends of pathological reporting in patients receiving neoadjuvant chemotherapy.

Methods: Representative sections from mastectomies and wide local excision were fixed in 10% formalin. Representative sections were processed and stained with hematoxylin and eosin to see the pathological changes that occurred after 4 cycles of neoadjuvant chemotherapy. Miller Payne system for classification of neoadjuvant chemotherapy treated breast cancers was used for reporting chemotherapy effect.

Results: 48 patients were selected for the study. Histological grades were 2 and 3. In all, 9(19%) patients had complete pathological response (pCR) in both breast and axilla Miller Payne Classification (grade 5). 39 cases showed partial response, out of which 5(12.5%) cases showed Miller Payne Classification grade 4, 10(21%) grade 3, 19(39.5%) grade 2 and 2(7.5%) cases showed Miller Payne chemotherapy effect grade 1. Only one case with pCR showed ductal carcinoma in situ. In 31 cases no lymphovascular invasion is identified and 7 cases showed lymphovascular invasion.

Conclusion: Neoadjuvant therapy is now being offered more commonly to patients with breast cancer and is becoming the standard of care. Pathologic assessment is of extreme importance and is still the gold standard as a prognostic marker for the patients, clinical trials and as an adjuvant for research studies. Pathologists have played and will continue to play an important role in providing this information. The role of pathologist is vital in standardizing classification scheme and developing new schemes.
POSTER PRESENTATION
Poster presentation: 1

Contribution of the gene Chek2 to the predisposition to breast cancer in Tunisian population

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Hereditary breast cancer HBC accounts for 3-8% of all breast cancers. Mutations in the BRCA1 and BRCA2 genes are responsible for up to 30% of the HBC cases. Previous studies in our laboratory estimated the prevalence of BRCA1 and BRCA2 gene mutations in breast cancer patients with affected relatives in Northern Tunisia. Contribution of the BRCA1 and BRCA2 deleterious mutations to breast cancer in these studies were confirmed in around 20% of the HBC Tunisian cases studied. Therefore, it has been suggested that mutations in other genes might account for significant proportion of HBC cancers in Tunisia such as in other populations.

The continued interest in the identification of new genes for hereditary breast cancer led to the description of CHEK2 gene that encode the "checkpoint kinase 2". This protein acts as a tumor suppressor that is activated in response to DNA damage and that interacts with several other proteins, including tumor protein 53, to halt cell division or induce apoptosis.

A founder allele in the CHEK2 gene (1100delC) has been associated with an elevated risk of breast cancer. This allele is responsible for the majority of CHEK2-associated breast cancers in women from Northern Europe. It seems to be rare in countries that are close to the Mediterranean.

The objective of this study is to evaluate the implication of CHEK2 gene in the occurrence of breast cancer in the Northern Tunisian population. To this aim, we investigated by nested PCR and DNA sequencing the contribution of CHEK2 gene mutations to familial breast cancer in Tunisia. Unrelated patients (20) who had at least one first degree relative affected with breast and/or ovarian cancer were analyzed and compared with 30 patients with non hereditary breast cancer and 30 healthy controls.
Poster presentation:2

Identification by HRM and COLD-PCR of “Hot Spot” mutations of the PIK3CA gene in patients with breast cancer.

Souassen Debouki

PI3K is a lipid kinase involved in the PI3K / AKT / mTOR pathway. Deregulation of this pathway by PIK3CA mutation is very common in various tumors. The analysis of the mutational status by PCR-HRM in exons 9 and 20 of patients with breast cancer and identification of mutations by COLD-PCR and sequencing showed that PIK3CA gene is mutated to 52.4% cases. We have demonstrated the presence of mutations E542K, E545K and E545A in exon 9 and two new mutations in exon 20 not described in breast cancer: H1047P and H1048Y. Simultaneous mutation at exon 9 and 20 has been observed in some samples.

We showed that the mutation was significantly associated with tumor size for sporadic and hereditary breast cancer. Statistical analysis showed that the mutation of patients in the sporadic form at the exon 9 is associated with an aggressive phenotype since it is correlated with tumor grade and the expression of the oncogene Her2. In addition to the inherited form, the mutation at exon 9 is correlated with age, tumor stage and tumor size and exon 20 with age. The mutational status of PIK3CA gene has a bad prognosis for overall survival of patients.
**Poster presentation: 3**

**Autophagy and breast cancer**

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Autophagy is a fundamental biological process that is involved in cell growth and plays a role in innate and adaptive immunity. In particular, autophagy-selective responses contribute to inflammatory bowel disease (IBD) neurodegeneration, and cancer. Autophagy is the mechanism by which cells consume parts of themselves to survive starvation and stress. This self-cannibalization limits cell death and tissue inflammation, recycles energy and biosynthetic substrates and removes damaged proteins and organelles, accumulation of which is toxic. This physiological mechanism is highly and finely regulated by about 30 genes described in literature, which are called ATG (Autophagy-related Genes). Those genes are involved in the three stages of autophagy which are initiation, elongation, and forming an autophagolysosome. The ATG protein, which is a key component of a large protein complex essential for autophagy, and polymorphisms within this gene have been reported to be associated with variety of diseases.

Taking into consideration that genes in the autophagy pathway play an important role in inflammation and immunity, and as a part of our ongoing research on the impact of genetic variants to the risk of inflammatory and sporadic breast cancer.

In order to investigate the contribution of ATG gene mutations to breast cancer in Tunisia, we explored the presence of an SNP (Single Nucleotide Polymorphism) in DNA samples extracted from 40 patients with breast cancer and 40 healthy women using PCR approach followed by sequencing the amplified fragments. The obtained sequences are analysed using bioinformatics tools to correlate the genotypes with the pathology.
IMPACT OF XPC GENE POLYMORPHISMS ON THE RISK OF COLORECTAL CANCER IN TUNISIAN PATIENTS

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Xenoderma pigmentosum group C (XPC) is a member of the nucleotide excision repair (NER) pathway. XPC protein is involved in the early recognition of DNA damage present in chromatin. The XPC gene is highly polymorphic and some of their variants are associated with several types of cancers. In fact many reports have shown that deficient DNA repair capacity resulting from polymorphic variation in this gene increased cancer predisposing factor.

The aim of this study was to evaluate the association of the A>C (rs2228001) and poly(AT) (Insertion/Addition) polymorphisms in XPC gene with colorectal cancer (CRC) risk in a Tunisian population-based case-control study.

The study was designed with 140 characterized CRC patients and 150 ages matched healthy controls. The XPC genotype was determined by PCR, PCR-RFLP and sequencing methods. Results were analysed by Epi-Info software.

Our results show that the homozygous genotypes, the CC and I/I respectively for the rs2228001 and poly(AT) polymorphisms increased risk of developing CRC in the Tunisian population.
Poster presentation:5

Stromal cell- Derived Factor-1 (SDF-1) polymorphism and its implication in sporadic colorectal carcinomas

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Stromal cell-derived factor-1, a CXC chemokine that play important roles in tumor growth, angiogenesis and metastasis. In the present study we aimed to investigate the allelic and genotypic frequencies of SDF1 3’A among patients with colorectal cancer (CRC) and evaluate risks of cancer outcome and progression. On the other hand, we tried to highlight the heterogenic expression of SDF1 in the colorectal cancer tissues according to different stages of disease progression.

Sixty colorectal cancer patients and one hundred cases of normal healthy control were investigated. Genotyping was carried out by PCR-RFLP method. The allelic frequencies are respectively [58,4%, 41,6%]; [22%, 78%] for A and G alleles in patients and controls. We underlined here, the significant difference of SDF1 3’A allele distribution between CRC patients and control group (p=0,0001) Conferring a high risk of CRC [OR= 0.141; 95%; CI= 0.065- 0.304]. On the other hand, the SDF-1 3’A seems to be associated with the early stages of disease according the TNM staging (p=0,015). Our immunohistochemical finding shows a significant association between the SDF-1 protein expression and its gene polymorphism. In fact, the membrane and cytomembrane over-expression were correlated with the presence of the mutated allele “A” whereas 92,4% of cases with negative expression are homozygous to the wild type allele “G”(p=0,000). In spite of the statistical analyses result (p=0,09), an association could be exist between SDF1 3’A allele and the invasion degree and that, will be clarified in the future with larger sampling.

The interesting finding of our work shows that SDF1 3’A mutation have a significant biological behavior in colorectal cancer progression, and on the basis of our results we conclude that the mutated allele of SDF-1 may be considerate as factors increasing the susceptibility of Tunisian colorectal cancer early stages of disease.
Poster presentation:6

The interactions of APC, beta-catenin and E-cadherin in Tunisian patients with gastric adenocarcinoma

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Gastric cancer (GC) is a significant worldwide health burden and is the second most common cause of cancer related death in the world although there has been a steady decline in the incidence and mortality risk of GC over several decades in most countries. Also, Gastric cancer is considered as a biologically heterogeneous disease involving numerous genetic and epigenetic alterations; however, previous studies have revealed that dysregulation of Wnt signal transduction plays an important role in human tumor development such as in gastric cancer. In this study, to evaluate the incidence of Wnt pathway activation and the predictive role of deregulated expression of APC, beta-catenin and E-cadherin in gastric adenocarcinoma, we investigated the expression of APC, beta-catenin and E-cadherin using immunohistochemistry, methylation specific PCR (MSP) and PCR amplification-sequence analysis in 82 cases of gastric carcinoma.

We showed that nuclear expression of beta-catenin was seen in only three cases and in the remainder of cases, the expression of beta-catenin was observed in both the cytoplasm and the membrane compartments of carcinoma cells (6% and 21% respectively). Mutation analysis for the three tumors with nuclear expression was showed that no mutations in exon 3 of beta-catenin. Abnormal expression of beta-catenin was observed more frequently in advanced gastric carcinoma (p=0.015). In multivariate analysis, the methylation of APC gene promoter showed a significant correlation with T-stage status (p=0.046) and Metastasis status (p=0.037). A significant positive association was found between expression of E-cadherin and membranous expression of beta-catenin (p=0.04). In univariate survival analysis, loss of membranous E-cadherin expression and abnormal expression of beta-catenin showed an association with poor prognosis. In conclusion, abnormal expression of APC, beta-catenin and E-cadherin occurs in considerable proportion of dysregulation of Wnt signal transduction that plays an important role in gastric carcinoma.
Poster presentation:7

Relationship between HER2 over-expression and clinico-pathological parameters in Tunisian gastric adenocarcinoma

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Background: Gastric cancer is a highly aggressive malignancy with poor prognosis and low survival rates. Although the TNM stage, Microscopic type and grading and patient’s age are the most important prognostic factor for this cancer, the variability of its prognosis within a clinical or pathological stage at presentation need a constant search for specific biological markers to identify subgroups of patients with more aggressive course disease. The purpose of this study was to investigate the relationship between HER2 over-expression and the clinico-pathological parameters in gastric adenocarcinoma. Methods: Tissue samples were obtained from surgically removed specimens of 100 patients with primary gastric carcinoma who underwent curative radical gastrectomy at Sfax university Hospital (Sfax-Tunisia) from January 1999 to December 2011. The expression levels in formalin-fixed paraffin-embedded gastric cancer were studied by immunohisto-chemistry and a statistical analysis of the correlation between HER-2 expression and clinico-pathological parameters was calculated. Results: There were 61 males and 39 females with a mean age of 58,72 years (range: 18-94 years). 51% of tumors were located in the antro-pyloric region, 41% in the body and 8% in the cardia. The mean size of tumors was 5.65cm (range: 2 - 19). Cancer was classified, according to the TNM Cancer Staging System of the American Joint Committee of Cancer, as stage I in 9 cases, stage II in 29 cases, stage III in 51 cases, and stage IV in 11 cases, respectively. Metastatic lymph nodes, distant metastasis and peritoneal carcinosis were observed respectively in 79%, 14% and 19% of cases. HER2 was over-expressed in 10% of cases. HER-2 over-expression were positively correlated with the age (p value: 0.038), distant metastasis (p=0.027) and with peritoneal carcinosis (p=0.01). Conclusion: More investigated are needed to identify specific biological prognosis markers of gastric carcinoma.
FIRST ASSAY OF MOLECULAR ANALYSIS OF GASTROINTESTINAL STROMAL TUMORS (GISTs) IN TUNISIAN PATIENTS

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Gastrointestinal Stromal Tumors (GISTs) are the most common mesenchymal tumors that may occur at any level of the digestive tract. Most GISTs harbour a mutation affecting the tyrosine kinase receptors such as the KIT or PDGFRA genes, whereas a small subgroup of tumors is wild type for mutations. These sarcomas are resistant to the conventional anti-cancer treatments methods and the tyrosine kinases inhibitors drugs remains the principle therapeutic sources for GIST patients. Detection of tyrosine kinases genes mutation has become an important laboratory assay for patients with GISTs because the results are useful to predict treatment response to many tyrosine kinases inhibitors drugs such as Sunitinib, Imatinib, Glivec or Nilotinib. In Tunisia the management and detection of mutations in GISTs were absent; the aim of this first molecular study is to evaluate and to determine the immunohistochemical expression status and mutational profile of KIT, PDGFRA, and also in BRAF and KRAS in order to introduce this routine assay as a useful method for clinicians to better improve the management of this disease.

Paraffin-embedded tumour sections of 18 GISTs were analyzed for KIT and PDGFRA expression by immunohistochemistry. For mutation analysis, exons 9 and 11 of KIT, exon 18 of PDGFRA, exon 2 of KRAS and exon 15 of BRAF were sequenced. This study shows that mutations in KIT and PDGFRA genes occur at similar frequencies in Tunisian patients as in other populations; and suggests that the same genes are at play in GIST, despite ethnic, geographical and environmental differences between countries. In addition, two patients arbour mutation in BRAF gene which suggests that mutation in this gene should be assessed in the future. Finally, we plan to increase the number of patients for better epidemiological and molecular characterization of GISTs in Tunisia.
Poster presentation: 9

The prognostic significance of K-ras and p53 mutations in colorectal carcinoma in Tunisian patients

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Objectives: Genetic alteration in K-ras and p53, have a pivotal role in colorectal carcinogenesis (CRC). Our group shown that somatic k-ras mutation occurs earlier than p53 in CRC and shown that K-ras and p53 mutations rarely occur in the same colorectal tumors. This suggests that mutations in these genes are on separate pathways to CRC development and may influence patient prognosis independently. In this frame, we investigate the presence or absence of K-ras and p53 mutations and their relationship with survival in Tunisian CRC patients. Methods: Mutation in codon12-13 of k-ras and exon5-8 of p53 were assayed respectively by PCR-RFLP and PCR-SSCP and then confirmed by sequencing in 55 Tunisian patients with CRC. Results: There were any association between p53 and k-ras mutation. No correlations has been detected according to the survival curves for p53 (p<0.05). Furthermore, patients with K-ras mutations had significantly poorer overall survival than patients without K-ras mutations (p<0.05). Conclusions: Our data indicate that the presence of K-ras mutations predicts poor patient prognosis in colorectal cancer, independently of tumors stage.
The prognostic significance of MDM2 and p53 mutations in colorectal carcinoma: Involvement and progression in Tunisian Population

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Objective: p53 and MDM2 have important role in the regulation of cell cycle. Their alteration may play an important role in the colorectal carcinogenesis (CRC). We evaluated the implications of MDM2 and p53 in the development of CRC, and provided their impact on risk and prognosis in Tunisian patients. Methods: p53 and MDM2, were genotyped for 167 patients and control and immunohistochemistry were performed. Results: MDM2 polymorphism studies showed a higher percentage of SNP309 in tumors but a very lower percentage of wild type, compared with the controls. The majority of samples with the SNP309 showed a positive expression of the MDM2 in tumor. Also, we found association between p53 expression and SSCP analyses and association between the MDM2 polymorphism and p53 mutation. SNP309 polymorphism and MDM2 overexpression was significantly related to higher mortality rates and we found a significant association between the MDM2 polymorphism and patient survival. Conclusion: Us and recent others studies are suggest the important role of the MDM2 which has long remained under the shadow of p53. We conclude that the effects of MDM2 SNP309 may be considered as a valuable prognostic marker to predict poor outcome for the Tunisian patients with CRC.
Discovery of carbon-nanotube genes’ target in human normal bronchial epithelial cells model

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Carbon nanotubes (CNTs) offer exciting opportunities for science and applications. In recent years, CNTs research has been established as a highly interdisciplinary field to exploit their outstanding features. Great interest has been generated in fullerenes in general, but especially in CNTs and carbon nanohorns as biologically compatible materials and drug carriers mainly because of their distinct architecture, hollow interior and cagelike structures. However, the small size, large surface area, and high reactivity of these materials are the main factors for potential toxicity. Moreover, CNTs will have wide-spread applications in many technological fields, thus worker/consumer exposure is likely to occur, posing emerging health concerns. Initial toxicological studies demonstrated that pulmonary deposition of CNTs causes acute pulmonary inflammation as well as chronic responses such as fibrosis. However, the mechanisms by which CNTs provoke susceptibility to toxicity and pulmonary inflammation is not clear. Thus, genome-wide monitoring of gene expression is important to understand the extent of CNTs effect. To this end, we have carried out a cDNA array analysis using Affymetrix probe-sets complementary to approximately 54 675 human genes, to monitor the levels of expression within aggregates of human normal bronchial epithelial cells treated with CNTs and their wild type cells. We identified a comprehensive list of genes that are differentially expressed between CNTs-treated and their control cells, the majority of them have been identified for the first time as targets of CNTs-effect. In addition to revealing the complex nature of the genetic changes after the accumulation of CNTs in human normal lung cells; we believe that our data can provide the possibility to use modified forms of CNTs as a potential therapeutic agent to treat lung cancer.

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Synthesis of sarcophine-copper (II) complex and its bioactivity in mice bearing Ehrlich ascites carcinoma

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Sarcophine (marine natural product)-copper(II) is a semi-synthetic complex, has been synthesized and characterized. In our previous work, we reported the potential affinity binding of sarcophine to DNA. In this investigation, we extended this work to study the bioactivity of sarcophine-copper(II) complex on mice bearing Ehrlich ascites carcinoma. In this study we report that sarcophine-copper(II) complex increases the affinity binding to DNA by phosphate backbone interaction and intercalation. Copper(II) complex was reported to improve sarcophine anticancer activity in mice bearing Ehrlich ascites carcinoma. The promising results obtained from the sarcophine-copper(II) complex interaction with DNA stimulated us to further substantiate the results with molecular modeling studies of interaction with human telomeric G-quadruplex DNA binding affinity.
Doxorubicin coupled to cell penetrating peptide promotes apoptosis in CHO cells by a mechanism involving c-Jun NH2-terminal kinase

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Doxorubicin (Dox) has demonstrated potent activity in treating malignant lymphomas but its therapeutic efficacy is hampered by induction of cardiotoxicity. This side effect is related to the ability of the drug to generate reactive oxygen species in cells. Previously, we demonstrated that coupling Dox to penetratin (Pen), a cell penetrating peptide, represent a valuable strategy to overcome drug resistance in CHO cells. In the present study, we evaluated the consequences of the conjugation of Dox to Pen in term of apoptosis induction. When tested on CHO cells, Dox-Pen generated a typical apoptotic phenotype but at lower dose that needed for unconjugated Dox. Cell death induction was associated with chromatin condensation, caspase activation, Bax oligomerization and release of cytochrome c. By using reactive oxygen species and c-jun NH2-terminal kinase (JNK) inhibitors, we prevented Dox- and Dox-Pen-induced CHO cell death. The chimeric soluble DR5 receptor that inhibits TRAIL induced cell death does not prevent Dox or Dox-Pen-induced cytotoxicity. These observations indicate that conjugation of Dox to cell penetrating peptide does not impair the ability of the drug to trigger cell death through activation of the intrinsic pathway involving c-Jun NH2-terminal kinase but could exhibit less toxic side effects and could warrant its use in clinic.
Evaluation of the antiproliferative activity of methanol extract and its fractions from red Mediterranean algae

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Cancer remains one of the most dreaded diseases causing an astonishingly high death rate, second only to cardiac arrest. The fact that conventional and newly emerging treatment procedures like chemotherapy, catalytic therapy, photodynamic therapy and radiotherapy have not succeeded in reverting the outcome of the disease to any drastic extent, has made researchers investigate alternative treatment options. In the present study we investigated in vitro the efficacy of methanol extract and its semi-purified fractions (F2-F3) of Padina sp for their antiproliferative effects by their potential antiproliferative activity using the MTT colorimetric method and clonogenic inhibition against three human cancer cell lines (A549, lung cell carcinoma, HCT15, colon cell carcinoma and MCF7, breast adenocarcinoma). Among the series the methanol extract and F3 exhibited interesting growth and colony inhibitory effects against the three cell lines in a concentration-related manner. These findings suggest that the polar active fraction F3 could contain a new antiproliferative compound(s). The purification and the determination of chemical structure of compound(s) of this active fraction are under investigation.

Keywords: antiproliferative activity; MTT colorimetric method; clonogenic inhibition assay; lung cell carcinoma; colon cell carcinoma; breast adenocarcinoma.
Background: Breast lymphoma, either primary or secondary, is a rare malignancy. Primary breast lymphoma (PBL) has a reported incidence ranging from 0.04% to 0.5% of all breast malignancies. It comprises less than 1% of all patients with non-Hodgkin’s lymphoma and approximately 1.7% of all extranodal non-Hodgkin’s lymphoma. Secondary breast lymphoma (SBL) has a reported incidence of 0.07%. It comprises 17% of all malignancies metastatic to the breast, making it the most common metastatic disease involving the breast. Most lymphomas involving the breast are of B-cell lineage, with only rare cases showing T-cell phenotype.

Purpose: We report three cases of breast lymphoma occurring in three women. Our aim is to highlight the clinical and the pathological features of this rare entity.

Results: The mean age of patients was 59 years, ranging from 36 to 80 years. One among the three women was known to have chronic lymphocytic leukemia treated with chemotherapy 10 years ago; the second women was known to have T-cell lymphoma involving lymph nodes and the pancreas while the third women had no previous medical history. The clinical presentation was a unilateral breast mass in two cases with an inflammatory skin appearance in on case, in the third case, the patient presented with gradual swelling and hardening of her both breasts. Histological and immunohistochemical examination of core needle biopsies revealed involvement of the breast with diffuse large B-cell lymphoma in two cases and T-cell lymphoma in one case.

Discussion: Malignant lymphomas of the breast are uncommon. They comprise less than 0.5% of all breast malignancies. The clinical presentation and radiological features of breast lymphoma and carcinoma are similar. Diagnosis is based on histological and immunohistochemical findings. Diffuse large B cell type is the most common histological subtype. Treatment includes chemotherapy and radiation. Prognosis of patients with PBL can range from 26 to 66% for 5 year survival rates; whereas for SBL, the prognosis is dependent on the staging of the primary disease.
**Poster presentation:16**

**Chronic lymphocytic leukemia in a patient with breast carcinoma: synchronous malignancy or chemotherapy-induced complication?**

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**Introduction:** The secondary leukemia following breast carcinoma is rare, and literature mostly describes acute myeloid leukemia and myelodysplastic syndrome associated with it. Chronic lymphocytic leukemia (CLL) following breast cancer is extremely rare with usually a long course of development. Nevertheless, it should be kept in mind that breast cancer may occur concomitantly with other malignancies especially CLL and that second malignancies occur with an increased frequency in patients with CLL, mainly because of the immune defects associated with this disease. We report an unusual case of CLL diagnosed 5 years after breast carcinoma treated with chemotherapy and radiation.

**Case report:** 5 years ago, a 60-year-old woman was histologically diagnosed with infiltrating ductal carcinoma of the left breast. After surgery, she received chemotherapy comprising 6 cycles of Epirubicin, Fluorouracil and Cyclophosphamides, combined with radiotherapy and Tamoxifène. Her hemogram at that time showed an increased level of total leukocyte count ($13 \times 10^9$/L) with 58% of lymphocytes at the differential leukocyte count, but big attention was not paid for it. On follow up, five years after the last cycle of chemotherapy, examination and radiologic investigations revealed a 2 cm right axillary lymphadenopathy while the right breast was free from tumor. Hematological investigations revealed hemoglobin 10g/dL, total leucocytes $31 \times 10^9$/L with a differential count of lymphocytes 81% and platelets $130 \times 10^9$/L. Excision of the lymphadenopathy was made. Immunohistochimical findings were consistent with CCL. **Conclusion:** Despite such potential benefits, adjuvant therapy may be leukemogenic. Such secondary leukemia is associated with poor prognosis and must be distinguished from primary leukemia which may also affect concomitantely patients with breast carcinoma. Through this case report, we emphasize that close and long hematological follow-up is essential in every case of breast carcinoma treated with chemotherapy.