

SCIENTIFIC PROGRAM

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Basic science and epidemiology

OCPS 1: Molecular profiling of breast cancer in the region of Marrakech

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Background: Breast cancer is the most common cancer. It is the second leading cause of death worldwide. A molecular classification of this cancer has been established recently. The molecular classification of breast cancer based on gene expression and the protein profile has distinguished five molecular groups: luminal A, luminal B, Her2/neu, basal-like and unclassified. The objective of this study is to classify 130 cases of invasive breast cancers in molecular groups and correlate the results with clinicopathological characteristics. **Methods:** Our retrospective study conducted in the Department of Radiation Oncology Hospital's Mohammed VI Marrakech, over 1-year since January 2012 until December 2012 with 130 patients. Tumors were analyzed histologically and classified according to a study by immunohistochemical groups: luminal A, luminal B, HER2+, basal-like and unclassified. **Findings:** Among the 130 tumors analyzed, 9 (6.9%) were classified as luminal A type, 40 (30.8%) as luminal B type, 27 (20.76%) as Her-2 type, and 48 (36%) as basal type. The luminal A subtype was correlated with low histological grade. The luminal B subtype is characterized by a higher histological grade with tumor size significantly higher than the previous type. In Her-2 subtype, tumor size was highest with a high rate of lymph node involvement. Finally, among basal like tumors, there was a high prevalence of histological types of poor prognosis, with a high SBR grade and a strong association with the presence of distant metastases. **Discussion and Conclusion:** This study confirmed the aggressive nature of the tumors and basal Her-2 compared with luminal tumors, which are characterized by morphological and clinical poor prognosis. Clinicopathological characteristics consistent with the molecular profile should therefore be considered as prognostic factors.

OCPS 2: Distribution and prognostic significance of molecular subtypes of breast cancer in Moroccan women

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Breast cancer is a complex and heterogeneous group of tumors in both their clinical behavior and their prognosis. Molecular classification of breast cancer is an important prognostic factor. In a retrospective study of 670 patients with breast cancer over 5 years (2009–2013) we classified breast cancer according to molecular markers (ER, PR, HER2) by means of immunohistochemical assays. The patients' average age was 48 years. The highest frequency was seen between 25 and 53 ans. Our study was characterized by an average tumoral size at the diagnosis of 5 cm, many nodular forms (51%). The histological study shows that most of tumours were invasive duct carcinoma (82.4%)

with high histo-prognostic SBR grade (level II – III: 86.3%). Axillary node metastases were more common (69.2%), blood vessel invasion has been shown in 42% of all tumors. The prevalence of the luminal A, luminal B, human epidermal growth factor receptor 2 (HER2), and triple-negative subtypes were 48.6%, 25.6%, 10.2%, and 15.6%, respectively. The triple-negative and HER2 subtypes were associated with poorer outcomes compared with the luminal A subtype among these Moroccan women. The characterization of these tumors and the establishment of a prognosis have significantly improved the therapeutic management and patient survival.

OCPS 3: Molecular profile of NSCLC in Morocco

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Introduction: In lung adenocarcinoma, the frequency of EGFR and KRAS mutations is ethnicity dependent with different proportion in white Caucasians and in Asians. The prevalence of these mutations among North Africans patients is unknown. The objective of this study was to report the frequency and spectrum of EGFR and KRAS mutations in a group of Moroccan lung adenocarcinoma patients. **Methods:** Tumor specimens from 137 Moroccan patients with lung adenocarcinoma were selected to determine frequency and spectrum of EGFR and KRAS mutations. Mutation detection techniques were polymerase chain reaction amplification and DNA sequencing. **Results:** The overall frequency of the EGFR mutation was 21%. Mutations were mainly detected in the exon 19 (69%), followed by exon 21 (21%) and exon 20 (7%), whereas mutations in the exon 18 were rare (3%). EGFR mutation rate was significantly higher in women and in never smokers. The overall frequency of the KRAS mutations was 9%. In the population with KRAS mutations, there was a trend towards more male and more smokers compared to patients with wild type KRAS. **Conclusion:** This is the first study to date examining the frequency and spectrum of EGFR and KRAS mutations in lung adenocarcinomas in North African and Arab populations. Some one fifth of lung adenocarcinoma tumors in Moroccan patients harbor EGFR mutations. This mutation frequency is higher than that found in whites but lower than in Asian population. KRAS mutation frequency in Moroccan patients was lower than white Caucasian population and comparable with the frequency observed in East-Asian population. Further studies, in larger numbers of patients, are needed to confirm these findings.

OCPS 4: Molecular characterization of *mlh1* and *msh2* germline mutations among colorectal cancer patients showing extinction of MLH1 and MSH2 proteins

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Introduction: Lynch syndrome, the most common form of hereditary colorectal cancer is mainly due to germline mutations

in a group of DNA mismatch repair (MMR) genes (MLH1, MSH2, MSH6, PMS1 and PMS2), Data on germline mutations of DNA MMR genes are available from different parts of the world in varying frequencies but no reports are available from Morocco. **Aim of the Study:** The objective of this study is to detect and study mismatch repair gene germline mutation among Moroccan patients with colorectal cancer, and identify the frequency of HNPCC in our population. **Patients and Methods:** In 200 prospectively recruited consecutive patients with clinically proven colorectal cancer. Immunohistochemistry staining was performed for MLH1 and MSH2 proteins on tumors from unselected patients with CRC. If IHC was abnormal, analysis of germline mutation of the 6 hotspot exons of the mismatch repair genes (mlh1 and msh2) was performed using direct sequencing. **Results:** IHC Staining and Clinical Findings: Among the 200 patients, only 10.5% (n = 21) showed abnormal IHC, negative staining for MLH1 was found in 66.6% (n = 14), negative staining for MSH2 was found in 44.4% (n = 7), one CRC was negative for both MLH1 and MSH2. None of these patients had previously been diagnosed with LS, the mean age at the diagnosis was 55.19 years (range, 28 to 81 years). Only eight (38.09%) of the probands were diagnosed at the age of 50 years. Regarding published family history criteria, none of the 21 patients fulfilled the Amsterdam criteria, and nine patients met the revised Bethesda Guidelines. Screening for Germline Mutations: At this time, DNA of 10 out of 21 patients were successfully sequenced, two types of variants were identified in MLH1 gene. Of these one was a missense mutation c.655 A>G in exon 8 among 4 patients, the second one was a c. -93G>A promoter polymorphism. However, no mutations were identified in any of the MSH2 exons among the 10 probands. **Conclusion:** The preliminary results of MLH1 sequencing among the 10 patients showed a high frequency of two polymorphisms (rs1800734) in the promoter region of MLH1 and (rs1799977) located in exon 8 of the same gene. In conclusion, more exons of both genes need to be screened.

OCPS 5: Molecular characterization of gastrointestinal stromal tumours: The experience of Hassan II University Hospital-Fez; Morocco

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Introduction: Gastrointestinal stromal tumours (GISTs) are the most common mesenchymal tumours of the digestive tract. Mutational analysis for KIT and PDGFRA genes is currently a standard practice to confirm the diagnosis of GIST, and to predict sensitivity to molecular targeted therapy. Centralization of mutational analysis in a laboratory enrolled in an external quality assurance program and with expertise in the disease is recommended. In the present study, we report our local experience to implement a standard gene analysis procedure for successful GIST mutation analysis and provide the preliminary results on the molecular characterization of GISTs in Moroccan population. **Materials and Methods:** Formalin-fixed paraffin-embedded (FFPE) GIST samples were used. Slide from each block was reviewed by experienced pathologists and the tumour area selected for DNA extraction. DNA was extracted from tumor areas containing at least 80% of tumor cells. Mutation of KIT exons 9, 11, 13 and

17 and PDGFRA exons 12, 14 and 18 were identified by Sanger sequencing of PCR products. Mutation nomenclature followed the recommendations of the Human Genome Variation Society (www.hgvs.org). Protocols and results were reviewed and validated in an international reference center (Institut Bergonié, Bordeaux, France). **Results:** 47 GISTs were analyzed for Kit Exon 11. Mutations were found in 23 cases (48, 9%); mainly deletions, followed by substitutions, and nucleotide insertions. Cases without mutations within exon 11 of Kit were analyzed for Kit exon 9 and PDGFRA exon 18; mutations were found in 3 cases and in 1 case respectively. Mutations localized within exon 9 were duplications. Further mutational analysis of KIT exon 13; 17 and PDGFRA exon 12; 14 will be investigated and reported. Single nucleotide polymorphisms (SNPs) in PDGFRA exon 18 (c.2472 C>T p.val 824 val) were detected in three cases. **Conclusion:** The genetic profile of Moroccan GIST patients is currently unknown and further investigation is required in this area of research. The present study is likely to contribute to the characterization of the molecular profile of GIST in Morocco.

OCPS 6: Molecular analysis of RET proto-oncogene in Moroccan patients with medullary thyroid carcinoma

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Purpose: Germline mutations of proto-oncogene RET are pathognomonic of hereditary medullary thyroid carcinoma (MTC). In this study, genetic analysis and familial testing of RET proto-oncogene in Moroccan families with MTC were performed. **Patients and Methods:** Thirty-one index cases with MTC and 115 of their relatives were included in this study. The entire coding region of RET was investigated by direct sequencing of PCR products. Once a mutation was identified, the target exon was sequenced in available relatives. **Results:** Seven distinct germline mutations of RET were identified in 45.2% (14/31) of probands. The most prevalent mutations were located at codon 634 (p.C634R/Y/F) and restricted to families with MEN2A (50% of the 14 mutation carriers, 7/14), followed by mutation at codon 918 (p.M918T) in all MEN2B cases (21.4%, 3/14), then by mutations at codons 804 (p.V804L/M) (14.3%, 2/14); and 891 (p.S891A) (14.3%, 2/14) detected in all patients with apparently sporadic MTC. Familial screening detected RET mutations in 19.1% (22/115) of the studied relatives; 36.4% (8/22) were found with MEN2A symptoms and 63.6% (14/22) asymptomatic. About 55% (12/22) were subjected to total therapeutic or prophylactic thyroidectomy. **Conclusion:** This is the first comprehensive genetic screening showing mutations spectrum of RET in MTC Moroccan patients with a predominance of mutations at codon 634. These results further support the necessity of genetic testing in MTC patients in order to provide earlier diagnosis and adequate initial treatment of these patients. This will moreover, contribute to the definition of a national policy to control this cancer in Morocco.

OCPS 7: Onco-viruses and human breast cancer

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Breast cancer is the most common malignancy in women worldwide; and metastatic breast disease is a major cause of morbidity and mortality in cancer patients. Recent studies pointed-out that certain onco-proteins of human viruses could convert non-invasive and non-metastatic human cancers to invasive and metastatic phenotype. On the other hand, it was estimated that 10–20% of human cancers, including probably breast cancer, are linked to virus infection especially onco-viruses. The most studied onco-viruses, in breast cancer, are mouse mammary tumour virus-like sequences (MMTV-LS), Epstein-Barr virus (EBV) and high-risk papillomaviruses (HR-HPV). In this presentation, I will discuss the presence and the role of these viruses in human breast carcinogenesis; especially, I will focus on the presence of HR-HPV and EBV in breast cancer in the Middle-East area and the role of E6/E7 and LMP1 onco-proteins of HR-HPV and EBV, respectively, which was largely explored by my group.

OCPS 8: Preclinical accelerated production of EBNA1 and LMP2-specific T-cells using gas-permeable rapid expansion cultureware for nasopharyngeal carcinoma adoptive immunotherapyWafa Khaali^{1,2,3,4}, Juliette Thariat^{1,5}, Laurence Fantin^{1,2}, Flavia Spirito^{1,2}, Meriem Khyatti³, El Khalil Ben Driss⁴, Sylvain Olivero^{1,2}, Janet Maryanski^{1,2}, Alain Doglio^{1,2}

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Nasopharyngeal carcinoma (NPC) is an Epstein-Barr virus (EBV)-associated tumor arising from the epithelium lining the upper part of the pharynx behind the nasal cavities. However due to this anatomical position, surgical resection of the tumor is rather limited and, despite good clinical efficacy of radiotherapy to treat tumor early stages, conventional treatments of advanced forms of NPC are commonly limited by distant refractory metastasis. Adoptive T cell therapy has been established as an attractive non-toxic immunotherapy for treatment of EBV-associated tumors. In this study, we have validated a rapid in vitro expansion protocol developed to generate large amount of EBV-specific T cells (EBV-CTLs) against the EBNA1 and LMP2 EBV proteins, described as major EBV latency II antigens in EBV-infected NPC cells. EBV-CTLs were generated from peripheral blood mononuclear cells (PBMCs) collected from NPC patients. PBMCs were stimulated with overlapping EBNA1 and LMP2 peptides and CTL amplification was rapidly achieved (16 days) using gas permeable culture devices (G-Rex). Interleukin-4 (IL4) and IL7 combination was shown to give the greatest overall expansion of specific EBV-CTLs, which interestingly were devoid of FoxP3+ regulatory T-cells. Our data suggest a simple and rapid method for a high-quality ex-vivo expansion of latency II EBV specific T-cells for the purpose of adoptive immunotherapy of NPC-refractory NPCs.

Key words: Adoptive T-cell therapy, G-Rex, latency II Epstein-Barr virus antigens nasopharyngeal carcinoma, T-reg

OCPS 9: Bioinformatics tools for detection of CXCR4 mutation and relationship to EGFR geneAbdessamad Amin, Ait Ssi Essaadia, Abdelmalek Hakmaoui¹, Mohammad Salah El-Nagger², Ahmed El-Tobgy², Amro S. Hanora³, Abdullah I. El-Falouji⁴

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CXCR4 is one of several chemokine receptors that HIV can use to infect CD4+ T-cells. While CXCR4's expression is low or absent in many healthy tissues, it was demonstrated to be expressed in over 23 types of cancer, including breast cancer, ovarian cancer, melanoma, and prostate cancer. Expression of this receptor in cancer cells has been linked to metastasis to tissues containing a high concentration of CXCL12, such as lungs, liver and bone marrow. In each cancer, there is a collection of somatic mutations, some of which create, control and/or direct the cancer phenotype. There are several statistical methods to identify the somatic mutations that are likely to be contributing to cancer pathogenesis. One way to determine which somatic mutations are probably driver mutations is by using bioinformatics tools that find genes that are somatically mutated more often than would be expected by chance or those that have a higher mutation rate than the background mutation rate in a cohort. In this study, different bioinformatics tools have been used to detect possible mutation sites on CXCR4 gene expressed in different cancer cells which could affect the binding of the antagonist to CXCR4 receptors. Our research results revealed 913 variant locations depending on updated Ensemble database (2014), 2 of 10 variant coding sequence locations have been selected to design the new primers. After that, BLAST (Basic Local Alignment Search Tool) have been used to compare individual DNA sequences associated with cancer to reference sequences that are known to be free of CXCR4 mutations, in order to investigate its relationship with the EGFR gene.

Key words: Bioinformatics, cancer, CXCR4, mutations, sequencer

OCPS 10: Thymoquinone nanoparticle formulations enhance its anticancer potentialIsabelle Fakhoury, Walid Saad¹, Kamal Bou Hadir², Regine Schneider-Stock³, Hala Muhtasib

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Thymoquinone (TQ) is a natural product with promising anticancer activity, but its development is hindered by its limited bioavailability. Drug encapsulation has been often used to overcome low drug solubility, bioavailability as well as non specific targeting. For this project, we aimed at synthesizing different TQ nanoparticles (TQ-NPs) using "flash nanoprecipitation", characterize them and test their anticancer potential in vitro in a panel of cells that range from normal to less aggressive cancers and highly aggressive and invasive cancer cell lines. We were successful at formulating four different stable TQ-NPs formulations that had an average diameter size between 45 and 130 nm. All TQ-NPs had also high entrapment efficiency and loading content that ranged between 72–83% and

70–90%, respectively. When testing TQ-NPs versus free TQ and blank NPs by MTT assay against normal MCF-10-A breast cells, MCF-7 breast cancer cells as well as the more aggressive MDA-MB-231 breast cancer cell line, we found that high TQ loading NPs enhance the antitumor activity of the drug when compared to free TQ while being less cytotoxic to the normal cell line. No significant cytotoxicity of the blank NPs was noted. The results generated from this project describe a new approach for the enhancement of TQ anticancer activity and therefore greatly contribute to the translation of this molecule to the clinic for various applications.

OCPS 11: Stability of the anticancer trabectedin

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Background: Trabectedin (Yondelis®) is an anticancer drug available for the treatment of soft tissue sarcoma and ovarian cancer. Currently, Trabectedin is prescribed as a continuous infusion for 24 h delivered via elastomeric pumps. These pumps allow patients to receive chemotherapy as outpatients, allowing them more independence and freeing hospital beds. The stability of trabectedin has only been tested for 30 h; therefore the drug cannot be prepared in advance and stored, since trabectedin might degrade before the start of treatment. The aims of this research are to determine the chemical, physical and microbiological stability of Trabectedin, under real life conditions over a 5 day period and to investigate the structure of any degradation products using mass spectrometry. **Materials and Methods:** Trabectedin for infusion has been prepared according to manufacturer's instructions and stored in elastomeric infusion pumps under varying conditions (ambient temperature and body temperature). Each pump has been subsampled once a day and the samples tested as follows: Physical Stability, Microbiological Stability and Chemical Stability. **Results:** Physical Stability - Particle formation has been assessed using visual and sub visual examination by optical microscopy and color change by UV/Visible light spectrometry. Microbiological Stability: The streak plating method has been used with agar plates incubated 37°C. Chemical Stability: Trabectedin concentration has been quantified using LC-MS/MS. The pH has been monitored. **Conclusion:** An LC-MS/MS Method has been developed according to the ICH guidelines. The stability of Trabectedin in elastomeric infusion pumps has been tested for 5 days.

OCPS 12: Differential effect of artemisinin against cancer cell lines

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The present study aims at defining the differential cytotoxicity effect of artemisinin toward P815 (murin mastocytoma) and BSR (kidney adenocarcinoma of hamster) cell lines. Cytotoxicity was measured by the growth inhibition using MTT assay. These in vitro cytotoxicity studies were complemented by the determination of apoptotic DNA fragmentation and Annexin V- streptavidin-FITC assay. Furthermore, we examined the in vitro synergism between artemisinin and the chemotherapeutic drug, vincristin.

The in vivo study was investigated using the DBA2/P815 (H2d) mouse model. While artemisinin acted on both tumor cell lines, P815 was much more sensitive to this drug than BSR cells, as revealed by the respective IC50 values (12 μ M for P815 and 52 mM for BSR cells). On another hand, and interestingly, apoptosis was induced in P815 but not induced in BSR. These data reveal an interesting differential cytotoxic effect, suggesting the existence of different molecular interactions between artemisinin and the studied cell lines. In vivo, our results clearly showed that the oral administration of artemisinin inhibited solid tumor development. Our study demonstrates that artemisinin caused differential cytotoxic effects depending not only on the concentration and time of exposure but also on the target cells.

Keywords: Antitumor activity, apoptosis/necrosis, artemisinin, cytotoxicity, synergism

OCPC 13: Alexandria Clinical Research Center "An International Collaboration by Egyptians for Egypt"

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Alexandria CRC is the first center for clinical trials in Egypt. It was established in July 2006 with the assistance of the University of Maryland, USA, under the twinning agreement between the cities of Alexandria and Baltimore. Trials are conducted according to ICH-GCP guidelines after approval of Research Ethics Committee (REC) of Alexandria Faculty of Medicine and Egyptian Ministry of Health (MOH). The center provides a full range of resources and services directly or in coordination with the institutional resources of Alexandria University Hospital: Research patient care, trained research personnel, lab facilities, statistical consultation, computer and data management support. Our mission is to enhance and expand the magnitude of high-quality and ethical clinical research and avoid exploitation of research subjects. Our vision is upgrading Alex CRC to be a Center of Excellence in Clinical Research. Our objectives are to foster more innovative, high-impact clinical trials and to streamline drug development activities enabling Egyptian patients to access the best medical care, to train local/regional researchers to conduct multinational trials and other health research projects and assume leadership within the MENA region. Also, to integrate all parties involved such as MOH, WHO, academia, pharmaceuticals, CROs and RECs in Egypt into one extensive network for clinical trials. Our research activities are investigator-initiated and industry-sponsored phase II, III and IV multicenter trials. Educational activities include training courses on GCP and research ethics for investigators, research coordinators and REC members, in English and French. Our future plans include obtaining CAP accreditation to the CRC laboratory, establishing a clean area for the optimum preparation of cytotoxic drugs, setting up database registry for clinical trials in Egypt, designing clinical research programs that meet the international standards, granting certified degrees in clinical research and assisting the establishment of other local and regional clinical research centers. Challenges regarding research subjects, investigators, REC, regulatory authorities, sponsors and local facilities as well as our ways to overcome them will be discussed.