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TAXOTERE este indicat, în asociere cu doxorubicină și ciclofosfamidă, pentru tratamentul adjuvant al pacientelor cu cancer mamar operabil, cu ganglioni pozitivi. TAXOTERE este indicat, în asociere cu doxorubicină, pentru tratamentul pacientelor cu cancer mamar avansat loco-regional sau metastazat, care nu au primit anterior tratament citotoxic pentru această afecțiune. TAXOTERE este indicat în monoterapie pentru tratamentul pacientelor cu cancer mamar avansat loco-regional sau metastazat, după eșecul tratamentului citotoxic. TAXOTERE este indicat, în asociere cu trastuzumab, pentru tratamentul pacientelor cu cancer mamar metastazat ale căror tumori exprimă în exces HER2 și care nu au primit anterior chimioterapie pentru boala metastatică. Cancer bronhopulmonar altul decât cel cu celule mici. TAXOTERE este indicat, în asociere cu cisplatină, pentru tratamentul pacienților cu cancer bronhopulmonar, altul decât cel cu celule mici, nerezecabil, avansat loco-regional sau metastazat, la pacienții care nu au primit anterior chimioterapie pentru această afecțiune. Cancer de prostată. TAXOTERE este indicat, în asociere cu prednison sau prednisolon, pentru tratamentul pacienților cu cancer de prostată metastazat, hormono-rezistent. Adenocarcinom gastric. TAXOTERE este indicat, în asociere cu cisplatină și 5-fluorouracil, pentru tratamentul pacienților cu adenocarcinom gastric metastazat, inclusiv adenocarcinom al joncțiunii gastroesofagiene, care nu au primit anterior chimioterapie pentru boala metastatică. Cancer al capului și gâtului. TAXOTERE în asociere cu cisplatină și 5-fluorouracil este indicat pentru tratament de inducție la pacienți cu carcinom cu celule scuamoase, al capului și gâtului, avansat local. **4.2 Doze și mod de administrare.** Doze recomandate. Pentru cancerul mamar, cancerul bronhopulmonar altul decât cel cu celule mici, cancerul gastric și cancerul capului și gâtului, începând cu o zi înainte de administrarea docetaxelului, dacă nu există contraindicații, se poate utiliza o premedicație cu un glucocorticoid pe cale orală, cum este dexametazona 16 mg pe zi (de exemplu 8 mg de 2 ori pe zi), timp de 3 zile. Profilactic se pot utiliza G-CSF pentru reducerea riscului de hemotoxicitate. Docetaxelul se administrează în perfuzie intravenoasă cu durata de o oră, o dată la 3 săptămâni. *Cancer mamar.* Pentru tratamentul adjuvant al cancerului mamar operabil, cu interesare ganglionară, doza de docetaxel recomandată este de 75 mg/m^2 administrat la o oră după administrarea de doxorubicină 50 mg/m^2 și ciclofosfamidă 500 mg/m^2 o dată la 3 săptămâni, timp de 6 cicluri. Pentru tratamentul pacientelor cu cancer mamar avansat loco-regional sau metastazat, doza recomandată de docetaxel în monoterapie este de 100 mg/m^2. Pentru tratamentul de primă linie, docetaxelul în doză de 75 mg/m^2 se asociază cu doxorubicină (50 mg/m^2). *Cancer bronhopulmonar altul decât cel cu celule mici.* La pacienții cu cancer bronhopulmonar altul decât cel cu celule mici, netratați anterior cu chimioterapice, regimul de doze recomandat este docetaxel 75 mg/m^2, urmat imediat de cisplatină 75 mg/m^2 timp de 30-60 minute. Pentru tratamentul după eșec al chimioterapiei anterioare cu compuși de platină, doza recomandată este de 75 mg/m^2 în monoterapie. *Cancer de prostată.* Doza recomandată de docetaxel este de 75 mg/m^2. Se administrează continuu prednison sau prednisolon 5 mg de două ori pe zi, pe cale orală. *Adenocarcinom gastric.* Doza recomandată de docetaxel este de 75 mg/m^2 în perfuzie intravenoasă cu durata de 1 oră, urmată de cisplatină 75 mg/m^2 în perfuzie intravenoasă cu durata de 1 până la 3 ore (ambele numai în prima zi), urmate de 5-fluorouracil 750 mg/m^2 pe zi, administrat în perfuzie intravenoasă continuă cu durata de 24 ore, timp de 5 zile, începând de la sfârșitul perfuziei intravenoase cu cisplatină. Tratamentul se repetă o dată la trei săptămâni. Pacienții trebuie să primească premedicație cu antiemetice și hidratare adecvată pentru administrarea cisplatinei. Trebuie să se utilizeze profilactic G-CSF pentru reducerea riscului de hemotoxicitate. *Cancer al capului și gâtului.* Pacienții trebuie să primească premedicație cu antiemetice și hidratare adecvată (înainte și după administrarea de cisplatină). Profilactic, poate fi utilizat G-CSF pentru a diminua riscul toxicității hematologice. Pentru tratamentul de inducție al carcinomului cu celule scuamoase, avansat local, inoperabil, al capului și gâtului (CCSCG), doza de docetaxel recomandată este de 75 mg/m^2 în perfuzie intravenoasă cu durata de 1 oră, urmată de cisplatină 75 mg/m^2 timp de 1 oră, în prima zi, urmate de 5-fluorouracil în perfuzie intravenoasă continuă cu 750 mg/m^2 și zi, timp de cinci zile. Acest regim terapeutic se administrează la fiecare 3 săptămâni, timp de 4 cicluri. După chimioterapie, pacienții trebuie să urmeze radioterapie. **4.3 Contraindicații.** Hipersensibilitate la docetaxel sau la oricare dintre excipienți. Pacienți care au anterior inițierii tratamentului un număr de neutrofile < 1500/mm^3. Pacienți cu insuficiență hepatică severă. Când sunt utilizate și alte medicamente în asociere cu docetaxel, se respectă, de asemenea, contraindicațiile acestora. **4.4 Atenționări și precauții speciale pentru utilizare.** În cazul cancerului mamar și cancerului bronhopulmonar altul decât cel cu celule mici, o premedicație cu un glucocorticoid pe cale orală, cum este dexametazona 16 mg pe zi (de exemplu 8 mg de 2 ori pe zi) timp de 3 zile, începând cu o zi înainte de administrarea docetaxelului, în absența contraindicațiilor corticoterapiei, poate reduce incidența și severitatea retenției de lichide, precum și severitatea reacțiilor de hipersensibilitate. În cazul cancerului de prostată, premedicația constă în administrarea de dexametazonă 8 mg, oral, cu 12 ore, 3 ore și 1 oră înainte de administrarea perfuziei intravenoase cu docetaxel (vezi pct. 4.2). **4.5 Sarcina și alăptarea.** Nu există informații privind administrarea docetaxelului la femeile gravide. Asemenea altor medicamente citotoxice, docetaxelul poate determina efecte nocive asupra fătului dacă este administrat femeilor gravide. De aceea, docetaxelul nu trebuie utilizat în timpul sarcinii, cu excepția cazului în care prezintă indicație fermă. Femei cu potențial fertil/contracepție: Femeile aflate în perioada fertilă care sunt tratate cu docetaxel, trebuie sfătuite să evite sarcina și, dacă rămân gravide, să se adreseze imediat medicului curant. **4.6 Efecte asupra capacității de a conduce vehicule și de a folosi utilaje.** Nu s-au efectuat studii privind efectele asupra capacității de a conduce vehicule sau de a folosi utilaje. **4.7 Reacții adverse.** Tulburări ale sistemului imunitar. Tulburări ale sistemului nervos: neurosenzoriale, neuromotorii. *Afecțiuni cutanate și ale țesutului subcutanat* Reacțiile s-au caracterizat prin erupții cutanate, inclusiv erupții localizate, în principal pe picioare și mâini (inclusiv sindrom mână/picior sever), dar și pe brațe, față sau torace, frecvent asociate cu prurit. Afectările unghiale grave sunt caracterizate de hipo- sau hiperpigmentare și, uneori, durere și onicoliză. ***S-a observat că alopecia este în evoluție*** *după* o perioadă de urmărire cu mediana de ***55 luni la 22 pacienți din 687 pacienți cu alopecie la sfârșitul chimioterapiei.*** *Tulburări generale și la nivelul locului de administrare* Reacțiile la nivelul locului de perfuzie au fost în general ușoare și au constat în hiperpigmentare, inflamație, eritem sau uscăciune a pielii, flebită sau extravazare și ectazie venoasă. Retenția de lichide include evenimente ca edemul periferic și, mai puțin frecvent, efuziunea pleurală, efuziunea pericardică, ascita și creșterea în greutate. Edemul periferic debutează de obicei la extremitățile declive și poate deveni generalizat cu o creștere în greutate de 3 kg sau peste. Retenția de lichide este cumulativă ca incidență și severitate. *Tulburări hematologice și limfatice* Rare: episoade de sângerare asociate cu trombocitopenie de grad 3/4. Pentru orice fel de reacții adverse, consultați RCP complet. **5. PROPRIETĂȚI FARMACOLOGICE. 5.1 Proprietăți farmacodinamice.** Grupa farmacoterapeutică: taxani, codul ATC: L01CD 02. **6. PROPRIETĂȚI FARMACEUTICE. 6.1 Lista excipienților.** Polisorbat 80, Etanol anhidru, Acid citric. **6.2 Perioada de valabilitate.** Flacon: 2 ani. După deschidere. Fiecare flacon este destinat unei singure utilizări și trebuie utilizat imediat după deschidere. După introducerea în punga pentru perfuzie. Din punct de vedere microbiologic, medicamentul trebuie utilizat imediat. Dacă nu este utilizat imediat, perioada de pastrare nu ar trebui să fie mai mare de 4 ore la temperaturi sub 25°C, incluzând timpul alocat perfuziei intravenoase la pacient cu durata de o oră. **6.3 Precauții speciale pentru păstrare.** A nu se păstra la temperaturi peste 25°C. A se păstra în ambalajul original, pentru a fi protejat de lumină. Pentru condițiile de păstrare ale medicamentului diluat, vezi pct. 6.2. **6.4 Instrucțiuni de manipulare.** *Prepararea soluției perfuzabile.* **NU UTILIZAȚI alt medicament care conține docetaxel, constând în 2 flacoane (concentrat și solvent) împreună cu acest medicament (TAXOTERE 20 mg/1 ml concentrat pentru soluție perfuzabilă, care conține numai 1 flacon). TAXOTERE 20 mg/1 ml concentrat pentru soluție perfuzabilă NU necesită diluție prealabilă cu un solvent și este pregătit pentru a fi adăugat în soluția perfuzabilă. 7. DEȚINĂTORUL AUTORIZAȚIEI DE PUNERE PE PIAȚĂ.** Aventis Pharma S.A., 20 avenue Raymond Aron, 92165 Antony Cedex, Franța. **8. NUMERELE AUTORIZAȚIEI DE PUNERE PE PIAȚĂ.** EU/1/95/002/003. EU/1/95/002/004. **9. DATA REVIZUIRII TEXTULUI.** Decembrie 2009. Informații detaliate privind acest medicament sunt disponibile pe website-ul Agenției Europene a Medicamentului (EMEA) http://www.emea.europa.eu. **Informatii pentru prescriere abreviate, bazate pe RCP decembrie 2009. Intotdeauna consultati RCP complet inaintea prescrierii medicamentului.**

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European Economic Interest Grouping

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First Conference of Romanian Division of IAP

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Biology and Therapy of Cancer Cell

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The Official Journal of „Prof. Dr. Ion Chiricuță" Oncology Institute

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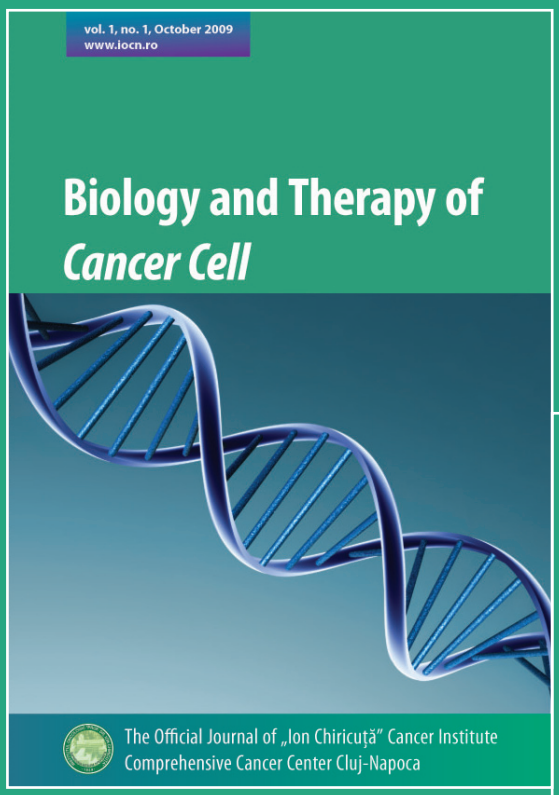
The „Biology and Therapy of Cancer Cell"Journal (the BTCC Journal) is the Official Journal of „Prof. Dr. Ion Chiricuță" Oncology Institute.

The „Biology and Therapy of Cancer Cell" Journal is an international peer-review, interdisciplinary journal published four times a year.

Each number shall have an invited Guest Editor to devote an issue on a specific scientific area.

The Editors of our journal welcome contributions from our readers.We seek high quality clinical and basic research reports from all aspects.

Topics shall cover gene discovery and therapy of cancer at clinical and preclinical stage (surgical oncology, medical oncology,radiation oncology, nursing oncology, supportive care and psychooncology), haematology, molecular mechanisms and personalised medicine, therapeutic options and novelties in cancer, cancer immunotherapy, DNA vaccines, RNA interference, post transcriptional control of gene expression, cell therapy, stem cells therapies, major discoveries in cancer therapy,cancer epidemiology,social and ethical and bioethical aspects of cancer. The journal will provide integrated view of modern oncology across all disciplines. Each discipline is represented in the Editorial Board.



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FIRST CONFERENCE OF THE ROMANIAN DIVISION OF IAP

"THE PATHOBIOLOGY AND MOLECULAR BIOLOGY OF TUMORS"

CLUJ-NAPOCA, ROMANIA
28-30 APRIL, 2010

ABSTRACTS BOOK

Contents

Building the European Area for Cancer Research

Claudio Lombardo

Special Assistant to the OECI President –
National Institute for Cancer Research of Genoa – Italy

Cancer is a major health challenge both worldwide and in Europe, where it is responsible for 25% of all deaths. The recent research progresses has led to an accumulation of knowledge on the molecular mechanisms of cancer and modern biology offers new possibilities to improve cancer prevention and care and clinical research is moving towards more complex multidisciplinary treatments. Integration of research and cancer care is therefore an increasing need to support personalized cancer medicine. By molecular pathology new subgroups of patients with unique sensitivities to treatments are identified, however, the impressive new information stemmed from the results of basic research has not yet met the expectations of patients in terms of clinical applications and benefit for public health. Despite the excellence of biomedical research and the significant financial support provided to cancer research in Europe, more concerted efforts are required. While competition remains a driving force for the discovery process, strong initiatives based on an integrated and managed approach as well as on national and international collaboration are essential for relevant advances. The "Lisbon Strategy" aims at making the European Union the most dynamic and competitive knowledge-based economy and the building the European Research Area (ERA) represents one of its fundamental elements. The 7th framework program is the main instrument specifically addressed to support the creation of such an area and it is a unique opportunity for the cancer community to promote excellence and support harmonization.

The OECI (Organization of European Cancer Institutes), built on cancer centers as members, implements the concept of comprehensiveness to guarantee the integration of cancer care, prevention, research and education. If centers share organization, create multidisciplinary cancer care and harmonize structures for translational cancer research, the problem of critical mass will find a solution contributing to the Lisbon strategy. From 2005 to 2007 the European Commission funded the Consortium EUROCAN+PLUS composed by scientists, Cancer Research Centers and Funding Organizations aimed to identify both the reasons for the European lack of competitiveness in translational research and the requirements for better coordination in cancer research. There were two main recommendations from EUROCAN+PLUS: the establishment of a European platform on translational cancer research and the need to involve National Funding Organizations in the launching of an ERA-NET on Translational Cancer Research with the purpose of better coordinating within Europe those research activities aiming at the benefit of the patients. Continuing education programs on translational research could be part of this coordination. OECI is strongly committed to support the outcomes of the EUROCAN feasibility study and after two years from the presentation of the EUROCAN final report to the Commission we can reasonable say that the objectives foreseen have been all achieved. In fact 3 European projects aiming to support the translational process entered the negotiation phase. We refer to the TRANSCAN ERANET on Translational Cancer Research, the TRAIN Co-fund, a 51/years/man mobility scheme for cancer translational research and the EUROCAN platform network of excellence. All these 3 initiative demonstrate how the Organization is contributing to the building of the European Area for Cancer translational Research.

Immunohistochemistry of the New Molecular Classification of Breast Carcinoma

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In recent years several microarray gene expressions, analysed by hierarchical clustering with fresh tissue RNA, have offered new genetic profiling of breast carcinomas. This technology has provided new subclassifications, dividing breast carcinomas into groups that facilitate a more precise prognostic and therapeutical approach. Nonetheless, the practical use of microarray technology is at present almost impossible in daily routine, not only because of the high cost, but also due to other technical problems such as the need to have fresh tissue available for the study [6].

Nevertheless, these advances have provided seminal information allowing the application of more accessible and cheaper methods such as immunohistochemistry in paraffin embedded tissues together with tissue microarray technology. Thanks to these two techniques, large series of tumors can be tested in single slides with a particular antibody, not only retrospectively, but also in prospective studies. Thus, carcinoma of the breast has been reclassified, not only based on its histology, but also supported by the positive or negative expression of a number of particular proteins of clinical, prognostic or therapeutic relevance. At present, several retrospective clinical analyses have validated this classification, and new studies underway will combine the microarray gene expression analysis with this methodology, providing a better and more comprehensive view of the biology of breast cancer [11].

At present four major types of breast carcinoma are accepted, and at least two more subtypes have been proposed [8]. These types are known as Luminal A, Luminal B. Basal-like and HER2/neu, and identified using a four–marker immunopanel: ER status, PR status, HER2/neu and Ki-67 proliferation index. The addition of CK 5/6 and EGFR allows the subclassification of the Basal-like subtypes into a triple negative and a core basal phenotype. Moreover an "Apocrine" (AR status and GCDP-15) close to the HER2/neu, and a "Claudin 1 low" stem-cell like phenotype have been proposed. Although this immunohistochemical-molecular classification has attracted wide interest, the validation at clinical level is still in progress.

The *Luminal A subtype* expresses ER and PR positivity while HER2/neu is negative, and display a low proliferative index (KI-67). This is the most common tumor in breast, mimicking normal luminal cells (positivity for luminal low weight cytokeratins 8/18) and genes associated with an active ER pathway. Histologically, it corresponds mainly to low grade carcinomas, such as low grade ductal, tubular, cribriform and lobular carcinoma of the WHO classification, and therefore presents low clinical stages and favourable prognosis.

The *Luminal B subtype* is the second more frequent breast tumor. It expresses ER, but PR status is low or negative and HER2/neu negative, while the Ki-67 proliferative index is high. It is also constituted by derivates of normal luminal cells (positivity for low weight cytokeratins 8/18) and has activated ER gene pathways, but simultaneously shows p53 mutations. The histological counterpart is mainly high grade ductal, NOS and micropapillary carcinomas. Their clinical outcome and prognosis is worse than luminal type A, but presents a good response to chemotherapy (TAC or FAC) and to the hormonal control. The clinical stages may be more advanced (stages II and III).

Basal-like subtypes constitute a low number of tumors (around 15 % of breast carcinomas correspond to this category) but they show a possible subdivision with prognostic implications [5] [4]. All basal-like carcinomas were characterized because the positivity for basal high-weight cytokeratins and specific myoepithelial cells markers (CK5/6, CK17, Caveolin1, Calponin1, P63) simultaneously lack

RE, PR and HER2/neu expression (triple negative) while Ki-67 is high, and also presenting p53 mutations and DNA repair defects. There is controversy regarding these groups of tumors because not all triple negative are genetically basal-like and not all basal-like genetically confirmed tumors display triple negative features. In addition, a group of basal-like carcinomas express EGFR and C-KIT positivity. This last variety would display additional worse prognosis when compared to the already known unfavorable clinical outcome and poor response to therapy of the basal-like category in which are included medullary, adenoid cystic and metaplastic carcinoma of the breast as well a small subgoup of high grade NOS of the WHO classification. In addition, this category is more frequent in *BRCA1* germline mutation carriers For some authors, the triple negative with additional negativity for CK5/6 and EGFR should be considered as unclassified tumors awaiting further information [11].

The *HER2/neu subtype* comprises carcinomas with definite positivity for the immunostaining with this antibody (clone DAKO, 3+) and confirmed with FISH or CRIST analysis. These tumors may belong to the luminal B type, but the majority correspond to the category of ER and PR negative tumors with a high Ki-67 positivity and occasional low CK 5/6 expression. They are very aggressive high grade ductal NOS carcinomas, nevertheless they respond well to the humanized monoclonal antibodies against HER2 or HER2 tyrosine kinase inhibitors (trastuzumab).

The *Apocrine type* is very infrequent and a great majority correspond to ductal NOS carcinomas with focal apocrine features and only exceptionally pure histological apocrine carcinomas are diagnosed, while apocrine metaplasia is common in benign ductal dysplasia and less frequent in in-situ carcinoma. Clinically they correspond to high grade tumors, are negative for ER and PR, and present AR positivity together with intense but focal GCDP-15 and occasionally HER2/neu 3+ .Their genetic profile has recently been partially identified [2; 3]. However, it is not clear if this group, as is also the case of the recently described "*Claudin1 low stem cell like carcinoma*" [7], configures particular clinical entities or should be included within any of the former indicated categories.

The *Normal cell breast-like type* carcinoma has been considered by some authors as another specific entity [10] but the personality of this tumor detected by means of unsupervised hierarchical clustering analysis by the Standford group [9] is not clear, because it mimics normal epithelial cells, and the histology and clinical significance has still to be determined [1; 11]

References:

1. Brenton JD, Carey LA, Ahmed AA, Caldas C (2005) Molecular classification and molecular forecasting of breast cancer: ready for clinical application? J Clin Oncol 23:7350-7360
2. Celis JE, Cabezon T, Moreira JM, Gromov P, Gromova I, Timmermans-Wielenga V, Iwase T, Akiyama F, Honma N, Rank F (2009) Molecular characterization of apocrine carcinoma of the breast: Validation of an apocrine protein signature in a well-defined cohort. Mol Oncol
3. Celis JE, Gromov P, Cabezon T, Moreira JM, Friis E, Jirstrom K, Llombart-Bosch A, Timmermans-Wielenga V, Rank F, Gromova I (2008) 15-prostaglandin dehydrogenase expression alone or in combination with ACSM1 defines a subgroup of the apocrine molecular subtype of breast carcinoma. Mol Cell Proteomics 7:1795-1809
4. Cheang MC, Voduc D, Bajdik C, Leung S, McKinney S, Chia SK, Perou CM, Nielsen TO (2008) Basal-like breast cancer defined by five biomarkers has superior prognostic value than triple-negative phenotype. Clin Cancer Res 14:1368-1376
5. Dent R, Trudeau M, Pritchard KI, Hanna WM, Kahn HK, Sawka CA, Lickley LA, Rawlinson E, Sun P, Narod SA (2007) Triple-negative breast cancer: clinical features and patterns of recurrence. Clin Cancer Res 13:4429-4434
6. Geyer FC, Decker T, Reis-Filho JS (2009) [Genome-wide expression profiling as a clinical tool: are we there yet?]. Pathologe 30:141-146
7. Herschkowitz JI, Simin K, Weigman VJ, Mikaelian I, Usary J, Hu Z, Rasmussen KE, Jones LP, Assefnia S, Chandrasekharan S, Backlund MG, Yin Y, Khramtsov AI, Bastein R, Quackenbush J, Glazer RI, Brown PH, Green JE, Kopelovich L, Furth PA, Palazzo JP, Olopade OI, Bernard PS, Churchill GA, Van Dyke T, Perou CM (2007) Identification of conserved gene expression features between murine mammary carcinoma models and human breast tumors. Genome Biol 8:R76
8. McCafferty MP, McNeill RE, Miller N, Kerin MJ (2009) Interactions between the estrogen receptor, its cofactors and microRNAs in breast cancer. Breast Cancer Res Treat 116:425-432
9. Perou CM, Sorlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, Pollack JR, Ross DT, Johnsen H, Akslen LA, Fluge O, Pergamenschikov A, Williams C, Zhu SX, Lonning PE, Borresen-Dale AL, Brown PO, Botstein D (2000) Molecular portraits

of human breast tumours. Nature 406:747-752
10. Sorlie T, Perou CM, Fan C, Geisler S, Aas T, Nobel A, Anker G, Akslen LA, Botstein D, Borresen-Dale AL, Lonning PE (2006) Gene expression profiles do not consistently predict the clinical treatment response in locally advanced breast cancer. Mol Cancer Ther 5:2914-2918
11. Tang P, Skinner KA, Hicks DG (2009) Molecular classification of breast carcinomas by immunohistochemical analysis: are we ready? Diagn Mol Pathol 18:125-132

Management of Soft Tissue Tumor Specimens in the Pathology Department: Practical Consequences for Diagnosis and Treatment of Patients With Sarcomas

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Soft tissue tumors are derived from nonepithelial extraskeletal tissue which includes fibrous tissue, adipose tissue, skeletal muscle, smooth muscle, blood vessels, lymphatics and peripheral nervous system. This tissues have usually a mesodermal in origin, with notable exception of peripheral nerves, which derive from neuroectodermal layer. The diagnosis of these tumors elicits precise responses to a specific set of questions regarding the malignant or reactive nature of the lesion, the type of differentiation which permits the assignment to a phenotypic category, the grade and the surgical margins of the resection. Many of these tumors have specific biomolecular or cytogenetic changes, or may appear in the context of genetic syndromes which need to be correctly identified. In order to satisfy all this requirements the soft tissue tumor specimens needs to be processed in a very systematic way, which will be detailed in this presentation.

Key words: Soft tissue tumors, differentiation, grading, cytogenetics, molecular biology

Lymphoma Diagnosis from Morphology to Molecular Genetics

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Malignant lymphomas are a heterogeneous group of diseases that are diagnosed and classified according to an integration of morphological, phenotypic, genetic, molecular and clinical features. This multidisciplinary approach has been very successful in recognizing a large number of distinctive entities with different pathogenetic mechanisms, biological behavior and management requirements. None of these parameters may be considered the gold standard for all diseases. In some entities the basic criteria would be the morphology but in others the information of other ancillary techniques is needed. A precise diagnosis in lymphoma requires the recognition of the specific disease, but also should provide information on aspects that may help to establish the prognosis of the patient and help to define the best treatment. The diagnosis of the major lymphoma categories has become highly reproducible among pathologists thanks to the use of the precise criteria defined in the WHO classification based on this multidisciplinary approach.

Morphology is the first step in lymphoma diagnosis and provides solid information but some architectural patterns and cytological aspects have overlapping features among entities making difficult the differential diagnosis. Phenotype is the second and a decisive step in the diagnosis of most entities defining the cell lineage of the tumor, stage of differentiation of the cells and certain specific features of the entities. The definition of prognostic parameters based on the immunophenotype has some limitations basically due to difficulties in the standardization of the techniques and evaluation of the results. Some lymphoma entities are characterized by specific genetic alterations. Conventional cytogenetics has been very useful to recognize these chromosomal aberrations but is difficult to apply in the routine clinical practice. Recently, the development of fluorescence DNA probes for in situ hybridization (FISH) has facilitated the identification of specific translocations and numeric changes in routine tissue sections and cytologies. The identification of the Epstein-Barr virus by In situ hybridization is also an important element in the diagnosis of some lymphoma entities. Molecular techniques are now a major help in establishing the clonality of some lymphoproliferative disorders. These techniques may be applied using DNA extracted from routine clinical samples but the time and type of fixation are crucial elements. This integrative and multidisciplinary approach allows the precise diagnosis of most lymphoproliferative disorders. However, the lymphoma diagnosis not infrequently confronts some cases that challenge our current concepts and working references. The current WHO classification has identified some provisional entities for which not enough information is available to recognize them as specific diseases. New observations and categories are opening new perspectives in lymphoma diagnosis. The progressive introduction of new genomic techniques in lymphoma research should help to understand better this interesting group of diseases and will provide new tools to be applied in the clinical practice. Open collaborations and discussions promoting consensus among all professionals involved in lymphoma managements are crucial for the advancement of our knowledge in this field.

Dendritic Cell Tumours

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The accessory cells of lymphoid tissues comprise specialised cells which interact with B- and T-cells in the immune response. Macrophage/histiocytes, interstitial dendritic cells, Langerhans cells (LC), veiled cells, interdigitating dendritic cells (IDC) and plasmacytoid dendritic cells (PDCs) are of bone marrow (BM) origin while follicular dendritic cells (FDC) and fibroblastic reticular cells (FRCs) are derived from mesenchymal precursor cells[1]. The BM derived cells share certain similar antigens which differ from those of the mesenchymal derived cells. These different cell types may rarely undergo neoplastic transformation and give rise to dendritic and histiocytic neoplasms. The main characteristics of these rare tumors and their updated classification are highlighted in this presentation.

Key words: Dendritic cell tumors, Langerhans cell tumors, Interdigitating cell sarcoma, Blastic plasmacytoid dendritic cells neoplasm, Follicular dendritic cell sarcoma

Pathobiology vs. Radiobiology in Cancer Treatment: Focus on Radiogenomics

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Knowledge of the genetic basis for individual radiosensitivity came from studies investigating rare cancer pre-disposing disorders and led to interest in measuring *ATM* mutations in relation to radiation toxicity. More recently it has been recognised that multiple cellular pathways are involved in the pathogenesis of radiation-induced normal tissue damage not only DNA damage recognition and repair but also cytokine and inflammatory response genes. Normal tissue radiosensitivity is now regarded as an inherited phenotype, and is considered to be a complex trait dependent on the interaction of multiple genes or gene products. This knowledge has led to interest in measuring genetic variation to predict a patient's likelihood of developing radiation toxicity).

The lack of correlation between *in vitro* tests and clinical response may partially be explained by the laboratory techniques used, intra- and inter-assay variability, inadequate patient follow up, cell types used, small study sizes and confounding factors61. For example, *in vitro* cell, chromosome and DNA damage assays of radiosensitivity cannot account for variability in cytokine response, tissue remodelling and collagen deposition in whole tissues. Nevertheless, the largest and one of the few prospective studies measured lymphocyte radiosensitivity in patients with carcinoma of the cervix, and found this to be an independent prognostic factor for the probability of toxicity-free survival. These findings underline a potential future role of genetic assessment of cancer patients to predict the individual treatment outcome.

Key words: radiosensitivity, damage recognition, radiogenomics

Circulating Tumor Cells Detection in Breast and Gynecologic Cancers

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Circulating Tumor Cells (CTC) may be considered the "leukemic phase" of the solid tumors and represent a prerequisite for the realization of distant metastases. The enumeration of CTC in peripheral blood of cancer patients has already clinical applications in breast, colorectal and prostatic cancer. It offers important information about the responsiveness of a given cancer to the treatment. It could also correlate with the survival (trials ongoing). This method has the potential to improve the understanding of the prognosis in an individual case and to show very quickly if a treatment is effective. Consequently, it enables the selection of the best therapeutic regimen, avoiding unnecessary treatment and potential toxicity. CTC could play the role of a "liquid biopsy" allowing the analysis for genetic mutations, which are more and more important in guiding the selection of the therapy. Another possible application could be a simple blood test to detect the presence of the CTC, indicating the presence of an unsuspected cancer in a given patient. Nevertheless, the identification of CTC remains a difficult process, necessitating multiple successive steps, the first one aiming at enriching these cells

in the sample. This presentation makes a quick review of the current and new developing techniques of CTC identification, highlighting some of the most promising clinical applications of the method in breast and gynecologic cancers.

Key words: Circulating tumor cells, detection methods, breast cancer, gynecologic cancer

Blood Angiogenic Markers as Noninvasive Diagnosis in Prostate Cancer

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Introduction:
Prostate cancer (PCa) is the most frequently cancer in the male population over the age of 50, in the worldwide. Early diagnosis of PCa could improve the cure rate for prostate cancer. We investigated in blood the value of some molecules involved in angiogenesis as potential noninvasive biomarkers for early prostate cancer diagnosis.
Material and methods:
Forty patients were included in the study. Thirty-four of these patients represent the typical patient population undergoing a prostate biopsy, with serum tPSA levels >4 ng/ml, and abnormal digital rectal examination results. The diagnosis for each patient from the study group: Prostate Cancer (PCa), Benign Prostatic Hyperplasia (BPH), and Chronic Prostatitis (CP), was established by evaluation of 10-12 core biopsies using Hematoxilin & Eosin staining. The remaining six patients were considered the control group with serum tPSA levels < 4ng/ml and normal digital rectal examination results. From each patient were obtained serum and RNA from blood, before any treatment or biopsy. The evaluations of blood molecules involved in angiogenesis were performed simultaneously using FAST Quant® array and PCR array technology. Statistical analysis was performed using SPSS software and $\Delta\Delta C_t$ method.
Results:
Our results show that there are significant serum concentration differences between the PCa, BPH, CP and Control groups for the following angiogenic molecules: KGF, angiopoietin-2, PDFG-BB and TIMP1 in sera from the patients evaluated. Also, our results show 5 genes to be significantly ($p<0.05$) up-regulated and 32 genes to be significantly down-regulated in the blood for prostate cancer vs control.
Conclusions:
Our data, using blood molecules involved in angiogenesis, could improve the early prostate cancer diagnosis in addition to the PSA value, abnormal digital rectal examination and pathological confirmation.

Key words: angiogenesis, blood, diagnosis, prostate cancer

Castleman's Disease

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Named after Benjamin Castleman (1956), Castleman's Disease(CD) or angiofollicular lymph node hyperplasia is a very rare disorder characterized by non-cancerous lymphocyte proliferation that may develop in the lymph node tissue especially in the mediastinum. CD is a heterogeneous disease that can be either localized or systemic (multicentric) with two distinct histological forms hyaline-vascular (HV) type and the plasma cell (PC) type. We report 2 new cases with an unusual clinical presentation and with some interesting histological feature and also we summarized the data from literature about pathogenesis and association with lymphoma.

Key words: Castleman' s disease, angiofollicular hyperplasia, lymphoma, HHV8

Molecular Staging of Prostate Cancer: the Role of the New Biomarkers

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Prostate cancer (PCa) is the most common noncutaneous malignancy in men and the second most common cause of cancer deaths representing an annual incidence of 679000 cases and 221000 deaths worldwide in 2008. The increasingly widespread testing for serum levels of prostate-specific antigen (PSA) has allowed for the increasing detection of PCa at early stages of development. As a result, prostatic adenocarcinoma has become a clinically heterogeneous entity, with some early carcinoma following and indolent clinical course, remaining confined to the prostate with little effect on overall lifespan, while other cases can lead to the development of lethal metastatic disease. Hence, in the diagnosis of PCa, the PSA test has enhanced the detection and awareness of this malignancy. Serum PSA levels have been widely used for diagnostic purposes for more than 25 years but false-positive and false-negative results are still common, especially in the so-called 'gray zone' (4–10 ng/ml), which represents a dilemma for discriminating CaP from benign prostatic hyperplasia, prostatitis and urethral manipulations which often increase PSA levels. Conversely, there is also a significant number of diagnosed PCa with a PSA below 4 ng/ml (estimated at 20–30%) resulting in undiagnosed disease.

In addition, despite the recent advances in treatment modalities, surgical, radiation, and hormonal therapies for PCa are not without complications, making the development of methods for distinguishing indolent cancers from their aggressive counterparts necessary to avoid excessive treatment that may lead to significant morbidity. Recent research has therefore sought to identify new biomarkers by which investigators can distinguish indolent PCa from those that go on to pursuer a more aggressive clinical course with the aim of optimizing the clinical management of the PCa patients. Among these new

biomarkers, special mention will be done to *TMPRSS2-ETS* fusion genes and *Prostate Cancer Antigen 3* (*PCA3*).

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Renal Cell Carcinomas in Patients with Radiation Exposure Following the Chernobyl Accident

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The Chernobyl Accident, which occurred in April 1986 in Ukraine, was the first event to pose the problem of chronic long-term effects of low-dose ionizing radiation (IR) in humans. More than 10 million people who live in radio-contaminated areas of Ukraine, Russia and Byelorussia are still exposed to low doses of persistent Cesium 137 (^{137}Cs) radiation, known to account for 90% of internal radioactivity, which is concentrated and eliminated through kidneys, and 90% of the more labile pool of ^{137}Cs which is excreted via urine[8,11].

Between the years 1986 and 2006, subsequent to the Chernobyl accident, the morbidity of malignant renal tumors in adults gradually increased from 4.7 to 9.8 per 100,000 of the total population (from 6.0 to 12.7 per 100,000 of the male population and from 3.6 to 6.6 per 100,000 of the female population, respectively) in Ukraine[9]. Conventional (clear-cell) is the most common subtype, accounting for 75% of all renal cell carcinomas (RCCs) [6].

Our group has observed a remarkable increase in tumor grade and proliferative activity of RCCs in Ukrainian patients following the Chernobyl accident[12]. In addition, a study focused on the development of *"radiation sclerosing proliferative atypical nephropathy"* in the peritumoral kidney tissue demonstrated a good correlation with the duration of radiation exposure[13].

The present study was carried out in order to examine the immunohistochemical (IHC) profile of a series of cell cycle regulators in conventional RCCs and peritumoral tissues from Ukrainian patients continuously exposed to low doses of IR. The comparative analysis of the identical molecular markers in the analogue tumors from the University Clinical Hospital, Valencia (Spain) was followed in the same study, using tissue microarrays (TMA) for their evaluation.

Chronic sustained low-dose IR is acknowledged to be a cause of DNA damage as well as an initiator of signal transduction cascades responsible for maintaining cellular homeostasis, radiation-induced genomic instability, and promoting interactions with neighbouring cells, known as the bystander effects[2,3]. Very few genes have been found to be consistently up-regulated by IR, although this includes genes associated with cell cycle arrest, growth control and cell signaling[7].

Our findings reveal strongly elevated levels of p53, and Ki67, in the Ukrainian cRCCs, in comparison with Spanish control tumors. Ukrainian cRCCs showed p53 protein over-expression in 22 (23%) of 97 cases, strongly associated with high Ki-67 overexpression, suggesting that p53 over-expression could also be involved in growth control in cRCCs. Although alterations in p53 expression have previously been found in human RCCs, controversy exists as to their frequency. Several studies reported an infrequent p53 expression in RCC samples (range 0 to 4%)[1,15]. However, other authors using IHC methods reported a 13.2% of p53 expression in RCCs[17]. Importantly, p53 over-expression in all cases of our study was associated with high grade and with strong mdm2 expression in the same cRCCs.

Although *p53* could remain wild type (WT) in the majority of RCCs, as previously published[18], recent studies have shown that WTp53 expression indicates an inactivated function of the WTp53, which could be deficient in its transactivation function[14]. These data could explain the strongly elevated

levels of mdm2 expression in 45% and 60% of the Ukrainian groups 2 and 3 respectively, especially in cases with co-expression of p53/mdm2. It is well known that mdm2 and WTp53 are involved in a negative feedback loop, a special regulatory mechanism that does not operate in unstressed homeostatic tissue[17]. In this loop, p53 is activated by $p14^{ARF}$as well as by mdm2 possibly in response to chronic long-term low dose IR exposure resulting in p53/mdm2/$p14^{ARF}$ molecular complex formation, a complex that is not seen in group 1 and is less frequent in group 2. These complexes result from the fact that p53 is not released from the nucleus thus activating other genes leading to proliferation, as is demonstrated by the high Ki67 index and/or apoptosis. Moreover, p53 in turn activates mdm2 which could inhibit p53 in two general ways. One way is by directly binding to p53 in order to block transcription and to stimulate degradation. Mdm2 can also promote the degradation of p53 by adding ubiquitin[4]. Our findings support a possible alteration of both p53 and mdm2 functions, which result in an insufficient feedback loop function associated with long-term low-dose IR exposure. Furthermore, in our cases, the mechanisms of WTp53 inactivation could possibly be explained by the deregulation of p53 regulatory proteins mdm2 and $p14^{ARF}$. Both proteins showed strong immunoexpression in the majority of the group 3 Ukrainian cRCCs, and could have been induced by long-term low-dose IR exposure[18].

Additionally, both cyclin D1 and cyclin G were over-expressed, independently of p53 status. It is of note, that the incidence and expression levels of cyclin G, and especially of cyclin D1, were higher in tumors from Ukrainian group 3. Cyclin D1 is a major regulator of cell cycle progression, especially at the G1 checkpoint, and is regarded as an oncogene that induces malignant transformation[16]. Cyclin G is one of the earliest p53 target genes to have been identified, and is also known as a negative regulator of the p53 gene during DNA damage[5]. Therefore, our results support the idea that the oncogenic role of cyclin D1 and cyclin G could possibly be enhanced under chronic long-term low-dose IR exposure[16].

In our opinion, the most important finding of the present study is the significant over-expression of $p14^{ARF}$ in cRCCs from patients chronically exposed to long-term low-dose IR. The *INK4a/ARF locus*, as mentioned above, encodes two cell cycle regulatory proteins: $p16^{INK4a}$ and $p14^{ARF}$, which enhance the growth-suppressive functions of the Rb and the p53 proteins, respectively. These proteins constitute a part of a network which is disrupted in most, if not all, cancer types[10]. The *INK4a/ARF locus* responds to stress signals, including IR, then limiting cell proliferation and modulating an oncogenic induced apoptosis[10]. Interestingly, high levels of $p14^{ARF}$ were associated with moderate and low p53 expression in 22 tumors, and with high mdm2 protein over-expression in the majority of cRCCs. These results suggest that p53 regulating elements mdm2 and $p14^{ARF}$ play a key role in the up-regulation of p53 activity under chronic long-term low-dose IR stress.

Our results indicated a direct correlation between the levels of peritumoral medullar radiation nephropathy associated with multiple areas of epithelial nuclear atypia in Ukrainian group 3 as well as Ki-67, cyclin G, mdm2, and $p14^{ARF}$ expression, with significant differences from the other groups. The moderate and severe radiation sclerosing proliferative atypical nephropathy was manifested by the strongest p53, mdm2, Ki-67, and $p14^{ARF}$ protein expression. In contrast, the Spanish peritumoral tissues showed decreased $p14^{ARF}$, mdm2, and cyclin G levels, with low intensity of staining. On the other hand, negative staining for p53, Ki-67, $p21^{WAF1/CIP1}$, and cyclin D1, was seen in groups 1 and 2, compared with the Ukrainian group 3 peritumoral tissues.

The fact that $p14^{ARF}$ and Ki-67 nuclear over-expression was detected in the endothelial cells of the tumor blood vessels in association with the constantly elevated levels of $p14^{ARF}$, mdm2, and cyclin G expression in vascular endothelial cells, fibroblasts and immune cells in the Ukrainian group 3 peritumoral tissues, suggest that chronic low-dose rate IR exposure also activates proliferative processes in a number of different target cells. It can be supposed that the microenvironment alterations of peritumoral tissue influenced by chronic low-dose IR exposure may potentially contribute to several facets of clear cell renal carcinogenesis including up-regulation of angiogenesis and tumor progression.

Taken together, these findings suggest that p53, mdm2, $p21^{WAF1/CIP1}$, Ki-67, cyclin D1 and cyclin G alterations in cRCCs from the Ukrainian patients living in the radio-contaminated areas may be involved in enhanced tumor progression in an environment of continuous low-dose rate radiation exposure. Critically, cell cycle transition alterations in damaged cells may enhance tumor promotion by increasing the probability that cells will occur with accumulating gene mutations.

References

1. Azzam EI, Toledo SM and Little JB. Stress signaling from irradiated to non-irradiated cells. *Curr Cancer Drug Targets* 2004; **4**; 53-64.
2. Eble JN, Sauter G, Epstein JI and Sesterhenn IA eds. Pathology and genetics of tumours of the urinary system and male genetic organs. *World Health Organization Classification of Tumours.* IARC Press, Lyon: 2004.
3. Fuhrman SA, Lesky LC and Limas C. Prognosis significance of morphologic parameters in renal cell carcinoma. *Am. J. Surg. Pathol*. 1982**; 6;** 655-663.
4. Gurova KV, Hill JE, Razorenova OV, Chumakov PM and Gudkov AV. p53 pathway in renal cell carcinoma is repressed by a dominant mechanism. *Caner Res.* 2004; **64;** 1951-1958.
5. Haitel A, Wiener HG, Baethge U, Marberger M and Susani M. Mdm2 expression as a prognostic indicator in clear cell renal cell carcinoma: comparison with p53 overexpression and clinicopathological parameters. *Clinical Cancer Res.* 2000; **6;** 1840-1844.
6. Kovacs G, Akhtar M, Beckwith BJ *et al.* The Heidelberg classification of renal cell tumours. *J. Pathol.* 1997;**183**; 131-133.
7. Morgan WF. Is there a common mechanism underlying genomic instability, bystander effects and other non-targeted effects of exposure to ionizing radiation? *Oncogene* 2003; **22;** 7094-7099.
8. Okeanov AE, Yakimovich AV. Incidence of malignant neoplasms in population of Gomel region following the Chernobyl accident. *Int. J. Rad. Med.* 1999;**1;** 49-54.
9. Pavlova L, Saydacova N. The state of urologic assistance for the population of Ukraine for 2003. In:*Ukrainian Ministry of Health. Annual reports of the Health Care in Ukraine*, Kiev, 2004; 108-109.
10. Perri MH. Mdm2 in the response to radiation Molecular *Cancer Res*. 2004**; 2;** 9-19.
11. Raes F, De Cort M, Graziani G. Multi–factoral nature of radioactivity deposition on soil after the Chernobyl accident. *Health Phys.* 1991; **61;** 271-282.
12. Romanenko A, Morell-Quadreny L, Nepomnyaschy V *et al.*. Pathology and proliferative activity of renal-cell carcinomas (RCCs) and renal oncocytomas in patients with different radiation exposure after the Chernobyl accident in Ukraine. *Int. J. Cancer* 2000; **87**; 880-883.
13. Romanenko A, Morell-Quadreny L, Nepomnyaschy V *et al.* Radiation sclerosing proliferative atypical nephropathy of peritumoral tissue of renal-cell carcinomas after the Chernobyl accident in Ukraine. *Virchows Arch.* 2001; **438;** 146-153.
14. Sejima T and Miyagawa I. Expression of Bcl2, p53 oncoprotein and proliferating cell nuclear antigen in renal cell carcinoma. *Eur. Urol.* 1998; **35;** 242-248.
15. Snyder AR, Morgan WF. Gene expression profiling after irradiation: clues to understanding acute and persistent responses? *Cancer Metastasis Rev*. 2004; **23;** 259-268.
16. Watanabe T, Katayama Y, Yoshino A, Komine C and Yokohama T. Deregulation of the TP53/p14ARF tumor suppressor pathway in low grade diffuse astrocytomas and its influence on clinical course. *Clinical Cancer Res.* 2003; **9**; 4884-4890
17. Ushida T, Gao J-P, Wang C *et al.* Clinical significance of p53, mdm2 and bcl2 proteins in renal cell carcinoma. *Urology* 2002; **59;** 615-620.
18. Vasavade SP, Novick AC, Williams BRG. p53, Bcl-2 and Bax expression in renal cell carcinoma. *Urology* 1998; **51;** 242-248.

Modern Approach in the Pathology of Prostate Adenocarcinoma

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More than 40 years after the inception of the Gleason grading system, it remains one of the most powerful prognostic predictors in prostate cancer.

Gleason's original grading system, however, has undergone significant revision over the years, first by Gleason and his colleagues, and most recently at the 2005 International Society of Urological Pathology Consensus Conference. The consensus conference and subsequent articles proposing further modifications have helped pathologists to adapt the Gleason grading system to urologic practice in a uniform manner. The changing definitions of Gleason pattern 3 and 4 prostatic adenocarcinoma have tended to narrow the scope of pattern 3 carcinoma and widen the scope of pattern 4 carcinoma.

These modifications have had an important role in improving the inter-observer reproducibility of the Gleason system. There was a shift towards higher Gleason scores on biopsy and prostatectomy in

pathological practice after the ISUP consensus, although-there was no significant impact on the biopsy-prostatectomy Gleason agreement.

However, as many of these modifications are supported only by a few studies, long-term follow-up studies with clinical end points are essential to validate these recommendations. Whether these changes have a significant impact on the clinical treatment of prostate cancer remains to be seen.

Gastroenteropancreatic Neuroendocrine Tumors (GEP-NETs)

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Introduction

The presence of endocrine peptides secreted by the gut mucosa was known from the last century (Bayliss & Starling 1902). The term "carcinoid" was introduced to describe ileal tumors with distinct clinical evolution and morphology (Obendorfer 1907). His suggestion that the lesions represented a special cancer was heavily debated. However, as more and more small tumors were detected and described in the intestine, the neoplastic nature of the lesion was generally accepted (Saltykow 1912). In 1910, Huebschmann (1910) found similarities between tumor cells and cell that had previously been described by Kultschitzky in the crypts of Liberkuhn. Descriptions of patients suffering from diarrhea, cyanosis, cough and flushing, started in 1931 (Cassidy 1931). Subsequently, the clinical syndrome "carcinoid" was described as a consequence of a carcinoid tumor. The first report of a carcinoid syndrome, however, is attributed to Ransom (1890), who described a 50-year-old woman with severe diarrhea and a metastasizing tumor originating from small nodules in the ileum. However, it was not until 1953 that the carcinoid syndrome was related to the hypersecretion of serotonin from the carcinoid tumor (Isler and Hedinger 1953). In 1938, Feyter named "diffuse endocrine system" the compartment of cells that composed of argentaffin-positive and argyrophilic clear cells that gives rise to carcinoids. The histochemical identification of argentaffin and argyrophil cells provided the pathologists with fundamental knowledge to understand the nature of these tumors (Masson 1914, Grimelius 1968). In 1969, Pearse made a further step forward in characterizing these neuroendocrine cells, by naming them APUD cells, postulating that they derived from the neural crest was introduced to explain the origin of neuroendocrine cells (Pearse 1968). According to this theory, these cells have the common ability for amine precursor substances uptake and decarboxylation. The APUD theory was critical reappraised by Lechago (1987). He pointed out that not all endocrine cells (parathyroid) are capable of APUD, whereas some exocrine cells (Paneth cells) they are.

More than 14 different types of neuroendocrine cells have been recognized in the pancreas and the mucosa of the human gastrointestinal (GI) track (Solcia et al 1987). Some neuroendocrine cells retain their differentiation when proliferate and form tumors, while other types do not. Neuroendocrine tumors (NETs) are often easily recognized by histology alone; however, immunohistochemistry is necessary to confirm their nature.

The GEP-NETs were initially termed «carcinoids», for they considered as low malignancy tumors with organoid histological structure causing carcinoid syndrome. However, the typical carcinoid syndrome with flushing, blood pressure changes, endocardial fibroelastosis, etc. due to secretion of serotonin, histamine, tachikinines develops in a subset of such tumors representing only 1.6% of GEP-NETs. Therefore, the historical term "carcinoid" has gradually become inappropriate to encompass all neoplasms with neuroendocrine features (DeLellis et al 1984).

According to the recent WHO classification, they have reamed to «neuroendocrine» tumors and carcinomas and to date, the term «endocrine» tumors can also be used (Hamilton et al 2000, Solcia et al 2000, DeLellis at al 2004).

GEP-NETs originate from cells of the diffuse endocrine system resigned in the GI mucosa. Their incidence is approx. 2/100.000 for the GI in the USA, UK and Central Europe. Due to the evolution of diagnostic utilities, they are reported with increasing frequency. These neoplasms recapitulate the phenotypic characteristics of a parent cell type normally present in these organs. Despite their specific similarities, GEP-NETs demonstrate significant heterogeneity in their wide spectrum of biologic activities (cell differentiation, hormone production and secretion) and to variable extent in their clinical behavior and clinical outcome. Some of these differences can be explained on the normal physiologic interrelationship of various prototypic cell types, while others may be related to the underlying genetic/molecular alterations. For this reason, it is difficult to classify and predict their behavior with accuracy. Well-differentiated tumors are often suspected by histology alone; however, immunohistochemistry is necessary to confirm their nature.

The recent WHO classifications (Hamilton et al 2000, Solcia et al 2000, DeLellis at al 2004) were formed integrating biologic activities on neuroendocrine cells with the embedded concepts of cell differentiation, cell heterogeneity and anatomic site of tumor development. Several prognostic and predictive factors have been identified for different tumors. From the clinical perspective, the presence of regional or liver metastases represents the principal prognostic factor. The WHO clinicopathological correlations provide the clinicians with useful common parameters related to the assessment of tumor biology and clinical behavior.

The WHO classification take into consideration the cell differentiation and proliferation, the tumor size and level of infiltration, the presence of necrosis, vascular invasion and metastases, the neuroendocrine functional activity and other related clinicopathological associations. The tumor size and Ki-67 proliferation index represent essential cutoff parameters for histological separation, while a multifactor approach is used to separate tumors of benign or uncertain prognosis from those of malignant clinical behavior.

Neuroendocrine Tumors of the GI Track

According to the WHO classification, the NETs of the GI track are divided in four main categories:

A) Well-differentiated Neuroendocrine Tumor - «Carcinoid»

A low malignant tumor composed mostly of anastomosing trabecular or glandular structures. The cells are relatively uniform showing mild atypia or no atypia.

As a rule, nonangioinvasive tumors, restricted to the mucosa or submucosa, measuring <1 cm (or <2 cm in the appendix) and showing Ki-67 (clone MIB-1) < 2% are considered indolent.

Any deviation from these parameters indicates increased risk of clinical malignancy.

B) Well-differentiated Neuroendocrine Carcinoma - Malignant «Carcinoid»

This malignant tumor consists of epithelial cells with moderate atypia, forming solid, or trabecular nests. There is often deep invasion of the gut wall (muscularis propria or beyond) and metastases to regional lymph nodes or liver. The tumor size is usually >1 cm and the Ki-67 >2%. Perineural invasion or angioinvasion represent additional criteria of malignant behavior.

C) Poorly Differentiated Neuroendocrine Carcinoma - Small Cell Carcinoma

A frank malignant epithelial neoplasm composed of small to intermediate-sized tumor cells with moderate atypia. The tumor cells form solid nests or irregular aggregates. The tumors are usually large with deep invasion or destruction of the gut wall. They often show necroses and prominent angioinvasion, perineural invasion and local or distant metastases. The Ki-67 proliferation index is >20%. The p53 protein is frequently positive to both local and distant metastases.

D) Mixed Neuroendocrine-Exocrine Carcinoma

Rare bimorphous tumors, composed of prominent exocrine cells admixed with at least 30% neuroendocrine component. The biological behavior of the tumor depends on the aggressiveness of the exocrine component

Gastric NETs

Most gastric NETs are well differentiated, nonfunctioning arising from oxyntic mucosa in the corpus or fundus. There are divided into three distinct types: Type I associated with autoimmune chronic atrophic gastritis (A-CAG). Type II, associated with multiple endocrine neoplasia type 1 (MEN-1) and Zollinger-Ellison syndrome (ZES); the remaining befall to type III category of sporadic tumors. Gastrin-producing functioning tumors are rare representing approx. 1% of all gastric NETs.

Serotonin-producing NETs or carcinoids are immunoreactive for serotonin. In some of them, a small cell subset may express serotonin, gastrin, somatostatin, and pancreatic polypeptide. Only few

carcinoids produce histamine and 5-hydroxy-tryptophan. These lesions, when metastatic, produce "atypical" carcinoid syndrome. Sporadic NETs are usually more aggressive than those associated with A-CAG or MEN-1. They show higher Ki-67 LI, frequent p53 immunoreactivity and more frequent vascular invasion. Deeply invasive tumors show local or distant metastases.

Gastrin-producing NETs appear as single, often multiple mucosal-submucosal nodules.

Hyperplastic neuroendocrine cells are lesions are commonly found in hypergastrinemic conditions (types I and II). In addition, NETs are often associated with dysplastic or "precarcinoid" lesions. Therefore, dysplasia of neuroendocrine cells is considered precursor lesion. Hyperplasia can be simple, linear, micronodular and adenomatoid. In dysplastic lesions, the neuroendocrine cells are relatively atypical; they form micronodules, with a tendency for enlargement or fusion and may show microinvasion or newly formed stroma. Dysplastic lesions of more that 0.5 mm or invading the submucosa are regarded as tumors. The full spectrum of neuroendocrine cells, from hyperplasia to dysplasia and neoplasia can been observed in A-CAG and MEN-1/ZES.

Duodenal and upper jejunum NETs

Gastrinomas may be multiple, especially when found in association with the MEN 1 syndrome. Gastrinomas may be very small (a few millimeters), even when metastatic to regional lymph nodes. SMS-producing tumors are often large, deeply invasive and metastatic to regional lymph nodes. SMS-producing tumors often cause obstruction of bile flow and may be associated with Recklinghausen disease.

Ileac and large intestine NETs

Most are well-differentiated, often multiple. Only a minority of cases metastatic to the liver cause the typical "carcinoid syndrome" with flushing, blood pressure changes, endocardial fibroelastosis, etc. Most tumors of the rectum and distal colon produce Serotonin or/and Pancreatic Polypeptide. Only tumors of the small intestine secrete enteroglucagon.

Appendiceal NETs

There are usually 0.5 cm or less in size. They arise in the mucosa and submucosa and infiltrate the wall. Most tumors confined to the appendix show benign behavior. Localization at the base of the appendix increases the risk of tumor spread. Few well-differentiated neuroendocrine carcinomas invade the mesoappendix with or without lymph nodes or distant metastases. Carcinoid syndrome is very rare; it is associated with massive metastases to the liver or retroperitoneum.

Pancreatic Endocrine Tumors

According to the WHO classification, the endocrine tumors of the pancreas are divided in three main categories:

A) Well-differentiated Endocrine Tumor

Tumors have a ***benign behavior*** when they are confined to the pancreas, they are nonangioinvasive, with no perineural invasion, they measure <2 cm and show Ki-67 (clone MIB-1) < 2%.

Tumors are of ***uncertain behavior*** when they are confined to the pancreas, but they have size >2 cm, or they show Ki-67 (clone MIB-1) > 2%.

B) Well-differentiated Endocrine Carcinoma

They are tumor of low grade of malignancy. The size is >2 cm and the Ki-67 >2%. Perineural invasion or angioinvasion represent additional criteria of malignant behavior.

C) Poorly differentiated endocrine carcinoma

These tumors are highly malignant. They often show necroses and prominent angioinvasion, perineural invasion and local or distant metastases. The Ki-67 proliferation index is >10%. p53 protein is frequently positive to both local and distant metastases.

Beyond WHO classification, novel grading and neuroendocrine specific grading/TNM classification/staging systems are proposed to provide tools for practical tumor histology assessment and effective patient classification. However, for the practicing pathologist, such classification/staging system requires complete clinical information for accurate histology report (Rindi et al 2007).

Somatostatin Receptors

Somatostatin receptor (sst) expression in NETs is of relevance to target somatostatin analogue-based diagnostic approach and treatment. The currently available somatostatin analogs bind preferentially sst2A and sst 2B (most widely expressed subtypes in GEP-NETs) and to a lower extent

types 3 and 5. The sst profiling in individual patients may be of relevance to better tailor the somatostatin analog-based treatment (Volante et al. 2008).

Immunohistochemistry for sst using currently available antibodies can be performed in most pathology loboratories on fresh and also archival tissue samples. The technique is a reproducible and easily accessible procedure (Thodou et al 2006).

Familial Syndromes

GEP-NETs may be associated with a MEN-1, von Hippel–Lindau (VHL), or rarely neurofibromatosis type 1 or tuberous sclerosis syndrome (Peren et al 2007, Anlauf et al 2007). Twenty to 60% of MEN1 patients suffer from a ZES, characterized by elevated fasting gastrin serum levels, a positive secretin stimulation test, and clinical symptoms such as recurrent peptic ulcer disease, gastroesophageal reflux disease, and occasionally, diarrhea (DeLellis et al 2004). Almost all tumors in MEN-1 patients show allelic loss of the wild-type allele. However, somatic mutations of the responsible gene located on 11q13 are detectable in a subset of sporadic tumors including 21% of those arise in pancreas. MEN-1 mutations are only detected in 7.7% of insulinomas and 8% of nonfunctioning pancreatic endocrine tumors. In contrast, a higher mutation rate was found in gastrinomas (37%), VIPomas (44) and glucagonomas (67%).

Somatic VHL gene mutations are very rare in sporadic pancreatic endocrine tumors, despite the high LOH rate on 3p25, indicating that the gene is less frequently involved than expected (Moore et al 2001).

References

1. Anlauf M, Garbrecht N, Bauersfeld J, Schmitt A, Henopp T, Komminoth P, Heitz PU, Perren A, Klöppel G: Hereditary neuroendocrine tumors of the gastroenteropancreatic system. Virchows Arch 451 (Suppl 1):S29–S38,2007.
2. Cassidy MA: Postmortem finding in a case shown on October 10, 1930 as one of abdominal carcinomatosis with probable adrenal involvement. Proc Roy Soc Med 24:920,1931.
3. DeLellis RA, Dayal, Y, Wolfe, HJ: Carcinoid tumors: changing concepts and new perspectives. Am. J. Surg. Pathol. 8:295,1984.
4. DeLellis RA, Lloyd RV, Heitz PU, Eng C (ed): World Health Organization Classification of Tumours, Pathology and Genetics of Tumours of Endocrine Organs. IARC Press, Lyon, 2004.
5. Feyrter F: Uber diffuse endokrine epitheliale organe. Barth JA, Leipzig, 1938.
6. Grimelius LA: A silver nitrate stain for cells in human pancreatic islets. Acta Soc Med Ups 73:243-270,1968.
7. Hamilton SR, Aaltonen LA (ed): World Health Organization Classification of Tumours, Pathology and Genetics of Tumours of the Digestive System. IARC Press, Lyon, 2000
8. Huebschmann P: Sur le carcinome primitif de l'appcndicc vermiculare. Rev med Suisserom 30:317-332,1910.
9. Isler P, Hedinger C: Metastasicrendes Dilnndarmcarcinoid mit schweren, vorwiegend das rechte Herz betreffendcn Klappenfehlern und Pulmonalstenose - eineigenartiger Symptomkomplex? Schweiz Med Wochenschr 83:4-7,1953.
10. Lechago J: The endocrine-cells of the digestive-tract. General concepts and historic perspective. American Journal of Surgical Pathology 11: 63-70 Suppl. 1,1987.
11. Masson P: La glande endocrine de l'intestin chez l'homme. C R Acad Sci (Paris) 138:59-61,1914.
12. Moore PS, Missiaglia E, Antonello D, Zamo A, Zamboni G, Corleto V, Falconi M, Scarpa A: Role of disease-causing genes in sporadic pancreatic endocrine tumors: MEN1 and VHL. Genes Chromosomes Cancer 32:177–181,2001.
13. Oberndorfer S: Karzinoide Tumoren des Dunndarms. Frankf Z Pathol 1:425-432,1907.
14. Pearse AG: The cytochemistry and ultrastructure of polypeptide hormone-producing cells of the APUD series and the embryologic, physiologic and pathologic implications of the concept. J Histochem Cytochem 17:303-313,1969.
15. Perren A Anlauf M, Komminoth P: Molecular profiles of gastroenteropancreatic endocrine tumors. Virchows Arch 451 (Suppl 1) S39-46,2007.
16. Ransom WB: A case of primary carcinoma of the ileum. Lancet ii: 1020-1023,1890.
17. Rindi G, Klöppel G, Couvelard A, Komminoth P, Körner M, Lopes JM, McNicol AM, Nilsson O, Perren A, Scarpa A, Scoazec J-Y, Wiedenmann B: TNM staging of midgut and hindgut (neuro) endocrine tumors: a consensus proposal including a grading system. Virchows Arch 451:757–76,2007.
18. Saltykow S: Beitrage zur Kenntnis der "karzinoiden Darmtumoren". Verb Dtsch Ges Pathol 15:302-307,1912.
19. Solcia E, Capella C, Buffa R et al: Endocrine cells of the digestive system. In: Johnson LR (ed). Physiology of the gastrointestinal track, 2nd ed, New York pp 111-130,1987
20. Solcia E, Kloppel G, Sobin LH: Histological typing of endocrine tumours. 2nd ed. Heidelberg: World Health Organization, 2000.
21. Thodou E, Kontogeorgos G, Theodossiou D, Pateraki M: Mapping of somatostatin receptor types in GH or/and PRL producing pituitary adenomas. J Clin Pathol 59:274-279,2006.
22. Volante M, Brizzi MP, Faggiano A, La Rosa S, Rapa I, Ferrero A, Mansueto G, Righi L, Garancini S, Capella C, De Rosa C, Dogliotti L, Colao A, Papotti M: Somatostatin receptor type 2A immunohistochemistry in neuroendocrine tumors: a proposal of scoring system correlated with somatostatin receptor scintigraphy. Moden Pathol 20, 1172–1182,2007.

Key words: carcinoid, familial syndromes, gut, neuroendocrine, pancreas, somatostatin receptors

Cryoglobulinemic Glomerulonephritis: Clinico-Bioptic Correlations

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Cryoglobulinemic glomerulonephritis (CGG) is a peculiar type om embranoproliferative glomerulonephritis, almost exclusively associated with type II mixed cryoglobulinemia (CG), composed of monoclonal IgMk with rheumatoid activity against polyclonal IgG.

In this study were included 15 patients, 8M and 7F, 42 to 75 years of age diagnosed in our center over the last 20 years with CGG. Of 14 patients tested for anti-HCV antibodies, 11 resulted positives. One patient had mixed type CG associated with primary Sjogren syndrome and two were considered as having "essential" CG. Immunologic evaluation included: determination of C3 and C4 serum levels, quantification and electrophoresis with immunofixation of cryoprecipitate. At the time of renal biopsy, 14 patients have had different levels and types of azotemia. All patients presented with macro/microscopic hematuria and 7with nephrotic range proteinuria. Renal biopsy with examination of renal tissue fragments in light and immunofluorescence microscopy were characterized by type I membranoproliferative lesions. In three cases with an apparent acute onset, hyaline intracapillary thrombi were seen. Electron microscopy revealed in two cases intense monocytic infiltration of the glomeruli and characteristic structurated electronodense deposits. Immunihistochemistry confirmed the presence of glomerular moncytes (CD68 positive cells) in one case.

In conclusion, the majority of our CGG cases were associated with HCV infection. Complex immunoserologic studies in correlation with renal biopsy findings, are the mainstay steps to confirm the diagnosis of CGG.

Key words: cryoglobulinemic glomerulonephritis, HCV infection, monocyte infiltration, structurated electronodense deposits

Application of Endosomolytic Cell-Penetrating Peptides for Nucleic Acid Delivery-Applications and Potential Implication for Cancer Treatment

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In the last few years an ever growing number of studies has implicated the use of nucleic acids as an efficient mean of targeting gene expression, especially for treatment of various types of cancer. The inherent sequence specificity of oligonucleotides (ONs) toward complementary RNAs and the fact that all RNAs are amenable for targeting have raised tremendous interest for their use, not only in basic science but also in therapeutic settings. However, most nucleic acids suffer from extremely low bioavailability

ensuing from their large size and hydrophilic chemical nature that make them essentially impermeable over cellular membranes. Therefore, numerous delivery systems have been exploited aiming at solving this issue.

A group of delivery peptides that seems very promising for this purpose is cell-penetrating peptides (CPPs). They have the advantageous feature of entering every single cell in a population in a relatively non-toxic manner, conveying various cargos ranging from small peptides to large plasmids. However, due to the negative charge of ONs and the cationic nature of CPPs, it is very complicated to generate covalent conjugates between the two entities and rather high concentrations of ONs are needed to obtain significant biological responses. The main impediment with the use of CPPs seems to be entrapment of peptides in endosomes following endocytosis. Therefore, exploring other vectorization methods using CPPs with improved abilities to promote endosomal escape is highly desired. We here present data on a series of novel, chemically modified CPPs with endosomolytic properties for efficient delivery of ONs (eg. Short interfering RNAs and splice correcting ONs) into various types of cells *in vitro* including e.g. various cancer cells and primary embryonic stem cells. Stable CPP/siRNA nano-particles rapidly enter entire cell populations resulting in strong and persistent RNAi responses, without associated transcriptome or proteome changes related to peptide treatment. Furthermore, strong RNAi responses are observed following systemic delivery in two different *in vivo* mouse models without any associated systemic toxicity. Finally, these particles can be dried as solid dispersions or subjected for gastric acid, without hampered activity *in vitro*, suggesting that they could be used for oral delivery. Finally, these peptides can be conjugated to tumor homing peptides in order to achieve tumor-targeted delivery *in vivo*.

Key words: cell penetrating peptides, endosomal release, cancer targeting, nucleic acids delivery

Lymphangiogenesis as a Potential Marker for Tumor Therapy

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Lymphangiogenesis is the process of new lymphatic vessel formation and has a major impact in the progresssion and metastasis of malignant tumors. Evaluation of lymphangiogenesis in cancer is nowadays based on the specific markers of the lymphatic endothelium. Induction and maintaining of this process is regulated by Prox-1 and VEGF-C/VEGFR3 axis. There are some question to be answered regarding tumor lymphangiogenesis: (1) How specific are the markers of the lymphatic endothelium? Nowadays, the most used is D2-40 that recognizes the formalin-insensitive epitope of podoplanin. (2) Which is the predictive value of the lymphatic microvascular density? Results for various types of carcinoma are still controversial. (3) The expression of VEGF-C and its cognate receptor, VEGFR3 always reflect a high risk for lymph node metastasis? Recent works showed that VEGF-C is not the only growth factor that stimulates lymphangiogenesis. (4) Which is the significance of the lymphatic endothelium-associated markers by tumor cells? It seems that this feature is associated with a more aggressive potential. (5) Lymphangiogenesis is similar in different molecular types of cancer? In breast cancer, only the HER2 and luminal types are significantly associated with increased lymphangiogenesis. (6) Which is the contribution of stromal cells in the progression of lymphangiogenesis? Recently, macrophages and mast cells were shown to play a role in the developing of new lymphatics. (7) And finally, lymphangiogenesis can be inhibited? Some of these questions were ansewered in part, based mainly on clinico-pathological and experimental studies. Others wait to be answered.

Microdissection from Carl Zeiss: a New Dimension in Sample Purity

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PALM systems allows contact-free manipulation and microdissection with lasers - the principle separation of the specimen occurs with a laser beam focused so precisely that a beam accuracy of less than 1 µm can be achieved. The selected specimen is removed with a software-controlled laser beam and catapulted by a laser pulse into a collection device. This procedure ensures that no unwanted elements reach the specimen. Because the laser in the process is only directed at the sample for about 1 ns, it does not transfer any heat. The process is completely contact and contamination-free and guarantees the best possible preservation of the material. Laser microdissection from Carl Zeiss provides an intelligent tool in molecular analysis at DNA, RNA and protein levels, as it highly improves sampling of cell-specific tissue. An innovation in the field of laser microdissection is the isolation of live cells from culture. Individual or small groups of cultured cells, even from primary cultures or stem cell preparations, can be used for direct molecular analysis or recultivation. This method has multiple fields of application either on fixed material, (cell analysis on microarrays, proteom analysis, plant research, forensics, chromosomes), or on live cells (stem cell applications, individual cell analysis, immunostaining of living cells). Basic principles as long as examples of concrete application are provided in this presentation.

Key words: contact free manipulation, laser beam microdissection, molecular analysis

Immuno-Histopathological Features in Human and Experimental Diabetic Nephropathy

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Roughly one third of diabetic patients (type I and II) sooner or later develop a specific nephropathy.

The diabetic nephropathy is clinically characterized by persistent albuminuria, progressive proteinuria, hypertension and renal failure. The specific pathologic lesion is the diabetic glomerulosclerosis due to a progressive increase of mesangial extracellular matrix and the thickening of glomerular basement membrane.

Characteristic light microscopy lesions are : a) glomerular hypertrophy due to the increase of filtration rate, b) a general thickening of glomerular basement membranes (GBM), c) expansion of the extracellular mesangial matrix either diffuse or nodular, forming the Kimmelstiel-Wilson nodules, d) aneurysmal peripheral glomerular capillaries due to mesangiolisis, e) glomerular hyalinosis, hyalinised capillaries or "capsular drops", f) almost 5% of patients develop extracapillary proliferation, g) arteriolosclerosis in the vascular pole, h) hyaline deposits in the efferent and efferent arterioles, i) thickened tubular basement membranes (TBM), j) interstitial chronic inflammation and fibrosis.

In immunofluorescence this pathologic complex is emphasized as linear labeling along the GBM with anti-IgG and anti-albumin antibodies.

The electron microscopy is providing further details of light microscopy lesions. Thus, the GBM thickening can be precisely appreciated according to the stage of evolution. This abnormality can be observed years before any clinical sign, therefore having a prognostic value.

All these features are important for the differential diagnosis with mesangioproliferative glomerulonephritis (GN), FSGS, GN with immune complexes, hypertension etc. In this respect, the following features are helpful: week or lack of mesangial cellularity, diffuseness of lesions, the linear labeling of GBM for anti-IgG and anti-albumin, GBM thickening and lack of immune deposits, arteriolar hyalinosis etc. The interstitial inflammation with myofibroblasts occurrence and the remodeling of TBM is a field still under investigation. We have noticed the association of myofibroblasts and TBM thickening.

The nodular pattern of diabetic glomerulosclerosis is a distinct entity which must be differentiated from amyloidosis, light chains deposition disease, immunotactoid GN, proliferative GN and the nodular idiopathic glomerulosclerosis.

The experimental diabetic nephropathy shows several common features with those described in humans and thus becoming of real help for understanding their pathogenic mechanisms. The experimental models show particular ultrastructural features mainly in GBM and nuclear glycogen deposits.

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The Role of the Biopsy in Pulmonary Pediatric Pathology

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The children's pulmonary pathology consist in entities who are characteristic only for children's (Congenital, Developmental, and Inherited Disorders; Acquired Non-Neoplastic Neonatal and Pediatric Disorders; Lung Neoplasms in Infants and Children) and entities who can affect any age (haemosiderosis, bronchial and bronchiolar pathology, pulmonary infections and metastatic tumors of the lung). This article debates only the first category of entities. We tried to permanently make reference to our personal experience in the field, unfortunately a quite poor one (only 61 bioptic examinations in the last 10 years). Our restricted experience referees to 27 sputum smears, 10 tracheo-bronchial aspirates, 6 pleural fluid smears and 14 surgical incisional pleuro-pulmonary biopsy. To compensate our poor experience in pulmonary biopsy in children's we tried to make reference when was needed to our much greater autopsic experience in the field.

Key words: pulmonary biopsy, children, anatomic pathology

Borderline Ovarian Lesions

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The behavior of ovarian borderline tumors and significance of various prognostic factors are unclear and difficult to evaluate because of inconsistencies and confusion in the literature.

The aim of the current review was to identify relevant information on ovarian borderline tumors by including all relevant reports from medical databases published in the latter years.

Issues such as the surgical pathological stage and the sub classification of extra ovarian disease into invasive and noninvasive implants are analyzed as well as survival.

We also discuss sensibility and sensitivity of ovarian cancer novel biomarkers, questioning whether gene profiling and proteomics could help differentiate between patients with metastatic ovarian cancer and primary ovarian carcinomas, and search for their potential impact on management. The new model of „two pathways" ovarian carcinogenesis based on clinical, pathological, and molecular genetic studies that may enable more targeted screening and therapeutic intervention to be developed is of major interest.In search for independent prognostic factors in patients with borderline tumors without residual tumour after primary surgery we have identified DNA-ploidy, international FIGO-stage, histologic type and patient age. Studies on other molecular markers have not yet uncovered a reliable prediction of biologic behaviour, however, there is hope that future studies of genetics and molecular biology of these tumours will lead to useful laboratory tests.Further questions to be addressed in our presentation include the following: Have patients with borderline tumours in general been over-treated and how should these patients be treated? How to define the high-risk patients? In which group of patients is fertility-sparing surgery advisable and, do patients with borderline tumours benefit from adjuvant treatment?

Validation of New Biomarkers for Cancer Therapy

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Introduction

Usual molecular biology and genetics tools for cancer research are limited to the analysis of one locus at a time. A cluster of genes that are regulated together by similar mechanisms can be identified by DNA microarray, and the functional relationships between them can discover new aspects of cancer biology. Ovarian cancer, one of the most aggressive malignant transformations, can be used to provide a model to demonstrate the current approaches to the molecular analysis of cancer.

Study protocol

Despite the exhaustivity of data to be interpreted microarray analysis is an important tool for the identification and validation of differentially expressed genes to increase power in clinical and biological studies across different sets of data. Recently, analysis approaches have been applied to large databases of microarray datasets to investigate molecular commonalities of multiple cancer types not only to find

the common molecular networks in tumor development but also to compare the datasets from each patient to other general cancer datasets to identify new sets of genes as possible markers. Till now this is not the case of ovarian cancer and more of borderline lesions which can bring information to be validated in a real time evaluation.

Techniques

Several researchers agree that microarray results should be validated. One commonly used method is quantitative reverse transcription PCR (qRT-PCR) to validate the expression profiles of the target genes obtained through microarray experiments. qRT-PCR is one of the most agreed method for clinical use, since it can be automated and performed on fresh, frozen or archived formalin-fixed, paraffin-embedded tissue samples.

Conclusion

The outcome of these analyses might accelerate the application of fundamental research discoveries into currently clinical practice through translational research and may have an impact on foreseeing the clinical outcome, predicting tumor response to personalized therapy, identification of new prognostic biomarkers, discovering new targets for novel therapies and providing further insights into tumor biology comprehension.

Key words: microarray, ovarian cancer, biomarkers, qRT-PCR analysis

Cytotoxicity and Cytogenetic Features of Oxaliplatin Resistant Colorectal Cancer Cell Line

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Introduction

Oxaliplatin (OX), a third-generation diaminocyclohexane platinum compound is currently used in the treatment of colorectal cancer. Despite its notable efficacy in primary tumor treatment, practically all metastatic cancers finally become resistant to this drug. Aiming to evaluate whether the OX resistant cells display a different behavior to this cytostatic drug, as compared to the sensitive ones, this study assessed cytotoxicity, apoptosis and potential cellular DNA damages production by OX.

Material and methods

In order to establish a stable cell line resistant to OX, the human colorectal cancer cell line (Colo320) was exposed to increasing doses of the drug, to the clinically relevant plasma concentration (2 μM/l OX). Four groups were selected for investigations: control (untreated) cells and 3 with different levels of chemoresistance (achieved by increasing pretreatment doses of OX up to: 1, 1,5 and 2 μM/l). All groups were subjected to additional doses of OX (0.1; 10; 50; 100 μg/ml) and cytotoxic effects, apoptosis rate and cellular DNA damage were evaluated.

Results

The IC50 values revealed the highest sensitivity to OX for the control group. Comparing the 3 OX pretreated groups we observed that cells exposed gradually to this chemotherapy drug were the most resistant to higher concentrations of drug, both at 24 and 48h exposure.

The apoptosis test showed comparable percentages of viable and apoptotic cells in all groups before the additional treatment with OX; administration of increasing concentrations of the drug caused differences in cells' viability and apoptosis rate, in correlation with doses and groups.

Comet assay revealed that control cells had the most affected DNA upon OX administration; in the pretreated groups the parameters that express DNA-damage showed an inverse relationship

between the level of the pretreatment OX-doses and the sensitivity of cells to the same chemotherapy drug.

Conclusions

Human colorectal cell line treated gradually with increasing concentrations of OX, reacted differently as compared to control, to the application of additional doses of OX. The pretreated cells displayed lower toxicity and cellular death, higher apoptosis/necrosis ratio and fewer DNA damages.

Key words: oxaliplatin, chemoresistance, citotoxicity, apoptosis, comet assay

Prediction of Clinical Outcome in Patients with Cervix Carcinoma by Pre-Therapeutic Evaluation of DNA Lesions

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Purpose

The aim of the study was to evaluate the *in vitro* radiosensitivity (RS) of tumor cervical cells and of normal cells from patients with cervix carcinoma and to correlate them with the clinical to radio-chemotherapy (RCT), in order to establish a predictive assay useful for the individualization of therapy in cervix carcinoma.

Patients and methods

A prospective randomized phase III trial with 2 arms was designed to include patients with stage IIB cervix carcinoma. 83 patients were included so far: 41 in the arm with RCT and 42 in the arm with RCT followed by surgery (RCT+SY). Tumor response was clinical assessed in patients with RCT alone, whilst in those with RCT+SY the tumor response was pathologically assessed. Normal tissues acute reactions were evaluated on the base of Common Toxicity Criteria (CTC vs. 2.0). Before treatment a tumor biopsy and a blood sample were obtained from each patient. The tumor cells from biopsies and the lymphocytes from blood were irradiated *in vitro* with 2Gy. *In vitro* RS of the cells was evaluated by Comet Assay and quantified by a lesion score (LS) determined before, immediately after and two hours after *in vitro* irradiation. The *in vitro* parameters of RS were correlated with clinical tumor response and with normal tissues toxicity to RCT.

Results

The tumor responses to RCT were categorized as: complete response (CR) and non-complete response (NCR). A CR was obtained in 75,9% of patients: 80,5% in the RCT arm and 71,5% in the RCT+SY arm. Basal level of DNA damage was lower for tumors with CR. The cellular DNA repair capacity was significantly different between tumors with CR and those with NCR to therapy: when the difference between LS at 2 hours after *in vitro* irradiation and the basal LS was higher than 0.10, there is the possibility to predict the complete or non-complete clinical response to RCT ($p=0.05$). Acute lower gastro-intestinal toxicities were not correlated with the *in vitro* RS of lymphocytes, but late toxicities seem to be.

Conclusion

Intrinsic RS of cervical tumor cells, especially their capacity to repair the radio-induced DNA lesions, is important for the fate of the cells after such treatments. Pre-therapeutic determination of cellular RS by Comet Assay could be a tool for prediction of the individual clinical outcome after RCT and for tailoring treatments in cervix carcinoma.

Key words: Radiosensitivity, cervix tumors, DNA lesions, Comet assay

POSTER SESSION

Histopathological Aspects in Uterin Cervical Cancer

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In Romania, uterine cervical cancer represents a major problem due to the fact that patients are diagnosed in the advanced stages of the disease, and screening procedures for the disease are only in an incipient stage.The study group consisted of 74 patients, aged between 34 – 68 years. All the patients were surgically treated and diagnosis was certified by paraffin histological exam. The specimens were examined by paraffin embedding. By identifying certain particular elements, the lesions were included in one of the histopathological subtypes of uterine cervical adenocarcinoma. In more than half of the cases (42 patients – 56.7 per cent) the histopathological subtype was endocervical mucinous adenocarcinoma. In 12 cases, histology showed the diagnosis of small cell non-keratinized carcinoma. In 4 patients where invasion was present, histology revealed villoglandular mucinous adenocarcinoma (3 cases) and clear-cell adenocarcinoma (1 case).

Conclusions:

1. Uterine cervical cancer represents a major health problem as, in Romania, it only comes second to breast cancer as far as malignant tumour induced female mortality is concerned. 2. The high incidence within the young women requires the development of screening programmes aimed at high-risk female population. 3. The size of the tumour is tightly related to the prognosis of the disease, mainly in patients undergoing surgical or radiological therapy. The size of the tumour in our study group ranged between 2 – 5 cm. 4. The histological type seems to have a predictive value both for the patients' survival and for the development of the disease.

Even if some authors have pointed out that in this respect there are no significant differences between adenocarcinoma and epidermoid carcinoma, others have reached the conclusion that surgically treated adenocarcinoma patients have very high relapse rates and very low survival rates.

Key words: Uterine cervical cancer, adenocarcinoma, epidermoid carcinoma

Histopathological Aspects in Thyroid Cancer

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Despite being rather a rare form of cancer, thyroid cancer is currently raising significant interest. Its incidence within all cancers has been estimated at about 0.75 – 1 per cent; however, when related to surgically treated thyroid disorders, its incidence has significantly increased up to 6 percent. The exact causes of thyroid cancer still remain unknown, but it is a known fact that people exposed to multiple radiation, both from the environment and following medical therapy, have a higher risk of developing this type of cancer. Most cases of thyroid cancer occur at the age group 35 – 50, with a higher incidence in women vs. men. The prognosis of the disease is depends on its histological type and the tumour's degree of differentiation; thus, the more undifferentiated the tumour, the greater the degree of local and distance invasion. Histological exam is the key to approaching a patient with thyroid nodules, and surgical treatment is instituted in all cases of thyroid cancer.The study was conducted on a group of 109 patients, hospitalized, diagnosed and treated in the II Surgical Department of the Clinical Emergency Hospital, Timişoara, Romania, between 2003 – 2008. The patients' age ranged between 18 –

74 years. All the patients were treated surgically and the diagnosis of thyroid cancer was confirmed by histopathological exam. The overall number of thyroid cancers recorded in this period was 109 cases, 98 of which were differentiated forms, and 11 undifferentiated forms. The most common form was papillary thyroid cancer; in our study group this form was found in 61 cases – representing 62.2 per cent of the total number of differentiated thyroid cancers (up to 75 per cent in literature); this form of cancer is the least aggressive form affecting mostly age extremes: below 40 and above 65. Follicular thyroid cancer – 22 cases, represented 22.4 per cent of the total number of undifferentiated thyroid cancers. Folliculopapillary thyroid cancer – 15 cases, represented 15.3 per cent of the total number of differentiated thyroid cancers.

Conclusions:

1. The incidence of this type of cancer increases in an alarming rate. The number of persons diagnosed with thyroid cancer has increased with over 10 per cent worldwide. 2. The most common type of tumour, the papillary carcinoma, has a peak incidence in the third and fourth decades of life. 3. Prognosis is directly dependent on the size of the tumour; tumours < 2cm have a very good prognosis. 4. The high incidence of these tumours in women, the female vs male ratio > 11, is much higher than shown in literature. 5. The overall macro- and microscopic alterations found in each case of thyroid cancer point to the necessity of thoroughly examining these alterations as they ultimately determine therapy and influence prognosis.

Key words: thyroid cancer, follicular thyroid cancer, papillary thyroid cancer

Histological Aspect in Colorectal Cancer

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The main histological type of malignancy is colo-rectal adenocarcinoma, which represents 90% - 95% of all primitive malignant colorectal tumors. Mucinous or colloid adenocarcinoma represents approximately 17% of tumors of large intestine, characterized by a large volume of extracellular mucin. Two to 4% of mucinous carcinoma cell carcinoma are represented by "signet ring cell" type, containing intracellular mucins. Colo-rectal cancer has an important representation in the Municipal Hospital General Surgery Clinic, Timişoara, with 331 cases treated in 5 years (2004-2008). Despite the progress of radio-chemotherapy in treating these cancers the central place is held by the surgery. In our study, particular forms (usually aggression) are most common (15.4%) compared with a maximum of 10% published by some papers. Colloid carcinoma represents 7.6% of all cases, being seen more often in men at all ages, except under 40 years age group. Signet ring cell carcinoma is more common in comparison with literature data. It develops predominantly in middle aged and young patients, younger than 40 years. The tumor was noted with increased incidence in women. Although considered a variety of undifferentiated carcinoma, medullary carcinoma is a distinct entity due to favorable outcome. In our study, medullary carcinoma was found in 1% of cases in patients aged between 60 - 80 years. It is more frequent in the colon. Our results confirm (as in the study dr.Pop Helena) the correlation between the degree of tumor differentiation and TNM stage. Patients with tumors in stage I (23 cases) were well-differentiated adenocarcinomas. Patients with tumors in stages II, III and IV (308 cases) carcinomas were associated with G3 and G4.

Key words: Colorectal adenocarcinoma, medullary carcinoma, cell carcinoma

Quantifying Invasion Groups Mediastinal Lymph Nodes in Lung Cancer

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Lung cancer is the leading cause of mortality due to malignancies, both for males and in females. Its prevalence is surpassed only by prostate cancer and breast cancer, but lung cancer mortality is still outranked by any other malignancy. Metastasis may occur via lymphatic, blood, or by direct contiguity, if the tumor is aggressive and take a break serous lung stationed locally. This aggression is characteristic of malignant tumors compared with benign ones who only grows, but extends not invade other healthy structures. If after carrying out investigations imaging results seem to suggest the presence of a tumor, most likely will make further investigations and analysis. To determine the nature of the tumor (malignant or benign) tumor biopsy will be done or it will extract a small amount of pleural fluid, where there pleurisy. Biopsy may be performed under guidance tomography to penetrate other organs not in the vicinity. American Thoracic Society classification of mediastinal lymph nodes ¬ best radio quantify anatomical topography of mediastinal lymph node groups is, therefore, by far the most widely used imaging practice. 78 studied a number of cases of lung neoplasms that had extensions in mediastinal lymphatic imaging station Radio Timişoara County Hospital investigated imaging and histopathology for diagnosis and therapeutic conduct.

Diagnosis, staging and posttherapeutic assessment of lung cancer was one of the most common indications for chest CT scan, and from these lymph nodes were detected in all cases. In 38 of lung cancer lymph nodes were isolated. This have set the stage quantification N1, N2 status in 14 cases and 26 cases in N3 stage.

Key words: Lung cancer, mediastinal lymphatic imaging, CT

Pathologic Anatomical Aspects of Malignant Ovarian Tumors

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Ovarian cancer is the fourth cause of death from cancer in women and in many countries. In our country it ranks second after the cancer of the cervix. The highest incidence rates of ovarian cancer have been reported in industrialized countries, paralelling the degree of urbanization and industrialization (except Japan, where the disease incidence rate is among the lowest in the world). At birth each girl has a 5-7% risk of developing life during an ovarian tumor and approximately 15% of these tumors are malignant. There are a variety of ovarian tumors, but over 90% are of malignant epithelial type. Ovarian cancer is diagnosed mainly in patients over 40 years. The peak incidence of this disease is in the fifth decade. Study group included 34 cases of malignant ovarian tumors treated laparoscopically; cases were hospitalized, diagnosed and treated in Department of Obstetrics and Gynecology "Bega", Timişoara. According to histopathological classification our group comprised: Border-line tumors → 6 cases (17.6%) (serous and mucinous, described by Dr. Stanciu Paul) G1 - tumors → 9 well differentiated cases

(26.5%), G2 - moderately differentiated tumors → 11 cases (32.3%) and G3 - 4 - poorly differentiated or undifferentiated tumors → 8 cases (23.5%). Ovarian tumors may arise from any cell component of ovarian tissues or their precursor cells. Hence the large number of histological types (68 types) of ovarian tumors. Ovarian cancer is the most dismal prognosis of all cancers, genital sphere with a survival at 5 years between 15-40%. Borderline tumors diagnosed and removed in time are perfectly curable (survival 100%). Prognostic factors are: residual volume, grading, FiGO stage, histological type, extraovarian extension.

Key words: Ovarian cancer, grading, FIGO stage

Endometrioid Carcinoma

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Endometrial cancer is the most common type of uterine cancer and constitutes about 95% of all malignant lesions of this anatomical segment. There are two different types of endometrial cancer. The first one develops during perimenopause, in women with history of exposure to estrogen, endogenous or exogenous. In these cases, the process begins as endometrial hyperplasia, progressing to carcinoma. These tumors "estrogen-dependent" are better differentiated and have a more favorable prognosis. The other type develops in cases where there is no evident source of estrogen stimulation. These spontaneous cancers are not associated with hyperplasia. They can grow on atrophic endometrium, are less differentiated and have an poor outcome. Cancers' non-estrogen dependent "are more common in older women (postmenopausal). The study group included 14 cases of endometrial cancer, the cases were hospitalized, diagnosed and treated in Department of Obstetrics and Gynecology "Bega", Timişoara. Endometrial cancer involved stage I - limited to uterus cancer: 8 cases, stage II - cancer spread to the cervix but not outside the uterus: 3 cases, stage III - cancer spread beyond the uterus but not beyond the pelvis: 1caz, stage IV - cancer spread beyond the pelvis, 2 cases. Endometrioid adenocarcinomas have a survival of 92% compared with 33% survival rate in the less common histological types (papillary serous, clear cell adenoscuamoase, undifferentiated). Histologically grade is closely related to prognosis. Cases with grade 3 tumors have frequent recurrences, with 5 times higher than those with grade 1 or 2. Miometrial invasion - increased muscle invasion is associated with extrauterine spread and recurrence potential growth. Survival rate decreases with increasing depth of miometrial invasion. Invasion of lymph nodes is an important negative prognostic factor.

Key words: Endometrial cancer, adenocarcinomas, invasion

Ovarian Tumors of Malignancy Limit

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First described by Taylor in 1929, they were named "semimalignant" tumors, carcinomas with low malignant potential (1963, 1971), borderline or tumors of limited malignancy (WHO 1973). After the

recognition of these new classification, 10%-l5% of all epithelial ovarian tumors were assigned to the category of borderline tumors. There are no reliable clinical, intraoperative or macroscopic criteria to corectly classify a tumour in the benign or malignant cathegory. The only reliable method of diagnostic is the microscopic histopathological examination of the tumor. In terms of anatomic and clinical diagnosis, the groups included cases of benign ovarian cystic tumors, suspicious and malignant. Our study group included 34 cases of malignant ovarian tumors treated laparoscopically; the cases were hospitalized, diagnosed and treated in Department of Obstetrics and Gynecology "Bega", Timişoara. In our study there were 6 cases (17.6%) of cystic borderline ovarian tumors (serous and mucinous). Mucinous borderline tumors have a better prognosis than serous. The surgical treatment for our group was as follows: younger women (4 cases aged 21-34 years) was performed unilaterally anexectomy careful and women in perimenopause (2 cases) and total hysterectomy bilateral anexectomy.

Key words: Bordeline tumors, microscopic histopathological, serous, mucinous

The Prevalence of Tumoral Skin Melanocytic Lessions in Children: A Retrospective Study on 58 Cases

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Introduction:
The tumoral melanocytic lesions are increasing in number in children due to a prolonged exposure to ultraviolet radiation. Although the skin malignant melanoma had a reduced incidence before puberty for a long time, being specific for adults, recent studies indicate an increasing number of these lesions with an increased frequency at pediatric age.

Material and methods:
The lot of study proceeds from the cases operated in the Clinical Children Hospital "St. Mary" Iaşi between 2002-2009. There were studied 55 cases of nevi and 3 cases of skin malignant melanoma.

Results:
From the 55 cases of nevi, 32 were in female patients (58,18%) and 23 in males (41,81%). From the 3 cases of melanoma, 1 was in females (33,33%) and 2 in males (66,67%). Most cases of nevi were registered in 2008 – 16 cases (29,09%), 2007 – 10 cases (18,18 %) and 2004 – 9 cases (16,36 %) and a reduced number in 2006 (6 cases – 10,90%), 2003 (5 cases – 9,09%) and 2002 (3 cases -5,45%). The nevi had a feminine predominance, being more frequent between 12-17 years of age (31 cases - 56,36 %), with a peak at 15-year olds. There were 43 cases of compound melanocytic nevi (78,18 %), with a slight feminine predominance, 5 cases of intradermic melanocytic nevi (9,09%) with a slight male predominance, 4 cases of jonctional (intraepidermic) nevi (7,27%), 2 Spitz nevi (3,63%) with equal sex distribution and 1 dysplastic nevus. From the 3 cases of melanoma, 1 case was before puberty and 2 cases after puberty, with a slight male predominance.

Discussions:
The increased number of pigmentary lesions could be explained by a prolonged and unprotected exposure to ultraviolet radiation of this group of the population and by a hormonal imbalance, the last factor being little studied in medical literature.

Key words: nevi, melanoma, prevalence

Useful Immunohistochemical Techniques for Elucidating Minute Atypical Glandular Proliferations in Prostate Biopsy Specimens – Preliminary Results

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Introduction:
The increased use of serum PSA determinations led to a substantial increase in the number of prostate biopsy (PB) specimens and also of cases containing minute suspicious prostatic proliferations. To elucidate these lesions, immunohistochemical (IHC) study is necessary but, unfortunately, the suspected foci are often lost during the paraffin block recutting. This paper presents the preliminary results obtained by applying two original IHC methods which saves the initial sections of interest.

Methods:
There were selected 2 groups of PB cases including, each of them, benign lesions and limited foci of adenocarcinoma. The IHC was performed, in both situations, by using one of the following primary antibodies: HMWCK, p63, P504S (Dako) and p63/P504S cocktail (Abcam). For the first group (8 cases) we tested the tissue protection IHC method (TPI) by immunomarking of one of the previously H&E - stained sections mounted on silanized slides. In the second group the IHC reaction was preceded by a process of tissue transferring from the initial HE stained slides (which contained minimum 2 sections) on special slides.

Results:
In all cases that were TPI proceeded, the interpretation of immunostained slides was optimal and the "protected" H&E – stained sections remained satisfactory for evaluation as well. Only in 1 from the 8 cases the transferred section was lost. In all the remaining cases the immunostaining was good or acceptable for an adequate interpretation.

Conclusions:
The above methods allow immunostaining on previously H&E-stained sections with a good quality of reactions. They represent viable solutions for the situations when the atypical small acinar proliferations are not present in deeper sections from the paraffin blocks and also an alternative to more expensive method of saving intervening unstained slides. Additionally, they can be successfully applied for other types of biopsies.

Key words: Prostate cancer, immunohistochemistry, minute atypical foci

Changing of the Immunohistochemical Profile of the Breast Cancer Cells During Chemotherapy: A Treatment Challenge

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Introduction:
The anti-tumor drugs may directly induce the expression of genes in cancer cells that could confer resistance or alternatively select clones with different immunohistochemical phenotype.
Material and methods:
During January 2009 and March 2010, 5 women with locally advanced breast cancer, diagnosed in City Hospital Timişoara, underwent pre-operative chemotherapy. Tissue fragments were fixed in formalin 4%, paraffin embedded, HE stained and subsequently submitted for immune reactions with ER, PR, Ki67 and HER2, using LSAB2 detection method and DAB visualization system. Neoadjuvant regimens were given, depending on physician's subjective experience. Mastectomy with axillary lymph node dissection followed, with histopathology and immunohistochemistry diagnosis of the residual tumor (if any).
Results:
2 cases (40%) diagnosed on core biopsy with infiltrative ductal carcinoma were basal-like, 2 (40%) luminal A, and 1 (20%) luminal B, with a wide range of Ki67 positiveness from 5% up to 60%. The response to neoadjuvant chemotherapy was good in 4 cases – 80%. 8 months after surgery, the case with the best pre-operative chemotherapeutical response (no residual breast tumor), presented liver metastases, which were biopsied in County Hospital Timişoara. The immunohistochemical profile of the metastases was apart from the initial core biopsy of the breast tumor (triple - whilst the breast cancer was triple +) that motivates a completely different therapeutical approach.
Discussions:
Response to medical therapy varies widely. This unpredictability makes the choice of the appropriate treatment difficult and so the selection of the initial regimen may need to be changed or abandoned. A possible breakthrough, surpassing the limitations of conventional methods is represented by the chemoresistance / chemosensibility testing assays.
Conclusions:
The change in the immune profile of breast cancer cells may be associated with either drug induction or drug selection of tumor cells during chemotherapy, resulting in relapses that determine a radical switch of adjuvant therapy.

Key words: Breast cancer, chemotherapy, immunohistochemical profile

Pleomorphic Sarcoma of Soft Tissue With Giant Cells, as Imunophenotipical Synovial Sarcoma

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Background:
Sarcomas with periarticular location, are relatively rare neoplasms. Synovial sarcoma (SS) is a morphologically distinctive entity, clinically and genetically. It can occur at any site. Usually well defined

SS is a mesenchymal spindle cell tumour with variable epihtelial differentiation, which can be extended along fascial planes and can invade bone.

Methods:

A woman aged 53, from whom was effectuated a soft tissue excisional biopsy; 4 months ago was made a prothetic hip implant, presented a complicated evolution: fever, joint pain, with a suppurative infection. She was treated for what was beleaved to be a latent infection (with staphiloccocus epidermidis), paraclinical: ESR-121, anemia. In his clinical history, 4 years ago, she suffered a neck femural fracture. The bone scintigraphy evidenced a hipercaptation in the tronhanterian area. The biopsy material was previously fixed in formalin, included in paraffin. We used HE usual stain as well as some special stains like VanGieson, H-PAS, alcian-PAS, reticulin and immunohistochemical markers.

Results:

The histology shows the aspect of a malignant tumor with increased cellular pleomorfism with fusocelular and round cells, which sometimes appear fasciculated, numerous multinucleated giant cells with abundent citoplasme, numerous mitoses: 6-10 HPF, focal extensive necrosis and hemoragic areas. Immunohistochemistry used a panel of markers: EMA, CD99, VIM, SMA are positive; CK, DESMINE repeatedly negative; CD68 -uncertain, unconstant results (negative/ positive when repeated)

Conclusions:

Most of our immunohistochemical markers have suggested a pleomorphic sarcoma, monophasic type, with giant cells, rhabdoid cells, numerous mitoses, (EMA, CD 99 positive), of poorly differentiated subtype. We have to mention in this case, that because of the short period of time ellapsed (Oct. 2009) after the total hip prothesis, it is difficult to determine the exact moment when appeared this malignant tumour (before or after prothesis). The correlation with the aspects of bone scintigraphy and CT examations are very important for establishing the precise diagnosis.

Key words: soft tissue tumor, pleomorphic sarcoma, synovial sarcoma

Relationship Between Cyclooxygenase-2 Expression and Angiogenesis in Patients with Gastric Cancer

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Aim:

To investigate the COX-2 expression in patients with gastric cancer and its relationship with angiogenesis and clinico-pathological features of gastric cancer.

Methods:

COX-2 expression and CD34-stained microvessel density (MVD) were detected by immunohistochemical methods in specimens from 61 consecutive patients with gastric cancer. The correlations among COX-2 expression, MVD and clinico-pathologic features were analyzed.

Results:

The COX-2 positive rate and MVD in gastric cancer were significantly higher than those in the normal gastric mucosa (57,4% vs. 4,9%; 38,7 vs. 12,5, all $P< 0.001$). The COX-2 positive rate and MVD in the patients with stage IV were significantly higher (65,4% and 42,2 respectively) than that in the patients with stage IA (33,3% and 35,5; $P<0.001$ and $P=0.035729$). The COX-2 positive rate and MVD in the pN3 cases were 87.9% and 42,4 respectively, higher than those in the cases without lymph node metastasis (32,1% and $P=0.031167$). The results also showed a strong correlation between COX-2 over-expression and depth of invasion ($P=0.0469$).

Conclusions:
COX-2 plays an important role in gastric cancer angiogenesis. COX-2 and angiogenesis induced by COX-2 contribute to tumor invasion and lymph node metastasis.

Key words: Gastric cancer; COX-2; angiogenesis

Immunohistochemical Expression of Ki-67, P53 and Cyclooxygenase-2 in Gastric Carcinoma: Correlation with Clinicopathological Factors and Survival

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Aim:
p53 mutation may contribute to upregulate cyclooxygenase-2 (COX-2) expression that is observed in malignant tissues. These molecules are involved in carcinogenesis by affecting tumor cell proliferation. The aim of this study was to examine the relationship between COX-2 or p53 expression and clinico-pathological factors including tumor cell proliferation in gastric cancer.
Methods:
COX-2 and p53 expressions were investigated with immunostaining, in tissue specimens obtained from 61 patients who underwent surgery for gastric cancer. The Ki-67 labeling index (LI) was counted by MIB-1 immunostaining.
Results:
COX-2 and p53 expressions correlated significantly with histological type after Lauren classification (P=0.00014; P=0.0212), tumor grade (P<0.001; P=0.039008), depth of tumor invasion (P=0.04269; P=0.02291), the presence of lymph node metastasis (P=0.031167; P=0.038264). There was no association between COX-2 expression or LI Ki-67 and survival. High LI Ki-67 (≥45%) were significantly correlated with age ≥61 years (P=0032068), histological type (P=0.006927) and tumor grade (P<0.001). The mean Ki-67 LI value of COX-2 positive tumors was significantly higher than that of negative tumors. The mean Ki-67 LI value of p53 positive tumors was not significantly higher than that of negative tumors. The mean Ki-67 LI value of both COX-2 and p53 positive tumors were significantly higher than that of both negative tumors.
Conclusions:
Our results show that COX-2 expression is associated with cell proliferation in gastric carcinoma

Key words: Ki-67, p53, Cyclooxygenase-2, gastric carcinoma

Antioxidant Effects of a Grape Seed Extract (GSE)

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Introduction:
Polyphenols are multifunctional derivatives and their antioxidant activity is mainly due to their redox properties which contribute to the absobtion and neutralization of free radicals. A potential source of polyphenolic compounds are the grapes (particularly red). Here we report the effects of GSE on lipid peroxidation and matrix metalloproteinases, MMP-2 and MMP-9 levels, in vitro.

Material and methods:
Cell culture materials: human lung fibroblasts (Hfl-1), human ovary carcinoma (Mls) and metastatic (M1/15) cells. Cell lysates: cells at densities of 10^6 were grown to confluence in 75 cm2 flasks. Doxorubicin(Dox) alone or in combination with GSE (37.5, 25, and 12.5 μEq GA/ml) were added to the medium and incubated for 24 h. Lipid peroxidation was determinated by measuring the MDA (malondialdehide) concentration and MMP activity was assayed using SDS-PAGE (zymography method). Statistical analysis was performed with a GraphPad Prism software program version 5.0. Comparasion between experimentes was made by 1-way Anova and Dunnett multiple comparasion test.

Results:
The influence of GSE on lipid peroxidation induced by Dox administration resulted in a dose-dependent decrease of lipid peroxides levels in normal cells (Hfl-1) versus a corresponding enhancenment in tumor cells (Mls). The effect of GSE on matrix metalloproteinases, MMP-2 and MMP-9 was investigated in normal cells (Hfl-1) and cocultures: normal (Hfl-1) and metastatic cells (M1/15). MMP-2 latent form was more responsive and significantly increased in cocultures versus normal cells. After GSE treatment its levels lowered bellow the control values in Hfl-1cells.

On contrary, MMP-9 was more sensitive to the treatment with xantin/xantinoxidase, a free radical generating system, when occurred the activated form of this protease. Both form, latent and activated, were decreased after GSE addition in a dose-effect relationship.

Conclusion:
These results demonstrate the antioxidant potential properties of the GSE, who is a good protector of normal cells in lipid peroxidation and matrix metallopreoteinases expression.

Key words: Polyphenols, doxorubicine, lipid peroxidation, metaloproteinasses

L-N-acetylcysteine Against Aminoglycosides' Ototoxicity in the Organ Cultures of Rat Cochlea

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Introduction:
Mammalian auditory cells are unable to regenerate after being damaged by toxic agents. Aminoglycosides are large scale antibiotics, extremely usefull for the treatment of several Gram-negative bacterial infections, but their use is limited by the extremely severe side effects like ototoxicity and nephrotoxicity. The aim of this study was to test whether L-N-Acetyl-Cysteine (L-NAC) can protect hair cells against Gentamycin induced damage *in vitro*.
Material and methods:
Cochlear explants from 1-4 days old rat pups were exposed to increasing doses of gentamycin. Half of the cochleas were pretreated for 24h with different doses of L-NAC. The explants were fixed and stained with phalloidin, and the intact hair cells were counted.
Results:
GM treatment resulted in the loss of sensorial cells in the organ of Corti explants in a dose-dependent manner. All doses of L-NAC offered significant protection ($p<0,001$) when added in culture 24h prior to GM. There was no significant difference between the level of protection offered by the different doses of L-NAC, both in the outer and inner hair cells.
Conclusion:
Our results demonstrate that L-NAC can protect cochlear cells against Gentamycin toxicity.

Key words: hair cells, cochlear explants, ototoxicity, otoprotection

Mechanisms of Apoptosis Induced by Oxaliplatin in Colorectal Cancer. Pharmacogenomics Study

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Introduction:
The goal of this study was to characterize the molecular pathways of apoptosis induced by oxaliplatin on colon cancer cells lines.
Materials and methods:
We used a colon tumor cell lines, colo 320, which was exposed to oxaliplatin for 24 respectively 48 hours. For the pharmacogenomics evaluation of apoptosis induced by oxaliplatin we used an apoptosis PCR-array technique. This is a new method of molecular investigation which permits simultaneous study of 84 genes involved in apoptosis. We considered the gene of interest whose of which the level of expression was 1.5 higher than the level of control gene. For bioinformatic analysis we used an interactiv

programme: Ingenuity Pathways Analysis (IPA). This is a network with hundreds or thausands of dirrect or indirrect interactions between mammal genes, at functional and physical level. The gene of interest were integrated in different networks with cellular biological functions.

Results:

Our study in vivo have showed different gene expressed in the two analysed moments of time. At 24 hours after administration some genes, like p53 and genes involved in immune and inflamatory response ware found overexpressed whereas the other genes involved in growth and cell prolifferation were suppresed. At 48 hours, there are activated molecular pathways involved in modulation of immune and inflamatory response and those involved in apoptosis (the intrinsec pathway by overexpressed of Bcl-2 gene and caspases).

Discussion:

The results obtained showed that oxaliplatinum act on cell lines by different pathways including the modulation of cellular cycle, imune response, cellular death and tumor transformation.

Conclusion:

By identyfing genes whose expression levels was modified at two differents times, the pharmacogenomics study brings new informations regarding the apoptosis of tumor cells induced by oxaliplatin.

Key words: Colon cancer, oxaliplatin, pharmacogenomics, apoptosis

Arsenic Trioxide Sensitizes Cancer Stem Cells to Conventional Chemotherapy – A Differentiation Therapy Model for Hepatocellular Carcinoma

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Objective:

The failure of existing treatments for liver cancer has been recently been atributed to the existence of cancer stem cells, difficult to kill using current drugs because of their chemoresistant properties as well as their ability to stimulate neoangiogenesis. The aim of the current study was to evaluate *in vitro* the antitumour efficacy of arsenic trioxide in combination with conventional chemotherapy, as proposed by the concept of "differentiation therapy" in anticancer research. Our results also show that low concentrations of arsenic trioxide lead to morphologic differentiation and differentiation-associated cytochemical features, such as incresed sensitivity to cytostatic drugs.

Methods:

Cancer stem cells showed enhanced chemoresistance to cancer drugs (Carboplatin and Doxorubicin) and had the ability to exclude Rhodamine123 dye, proving the existence of the multidrug resistance efflux pump. Arsenic trioxide was added prior to a tyrosine kinase inhibitor or to a slightly modiffied PIAF regimen with Capecitabine replacing 5-fluorouracil. We also compared both cancer and normal stem cell lines with the HepG2 non-stem liver cancer cell line to investigate the differences between differentiated and more anaplastic cells.

Results:
Initialy, cells had a high proliferative potential, even when cultured in medium supplemented with cytostatics, eliminated Rhodamine 123 immediately in culture and also formed spheroids in suspension. Low concentrations of arsenic trioxide lead to morphologic differentiation and differentiation-associated cytochemical features, like increased sensitivity to cytostatic drugs.

Conclusion:
Because there are fewer CSC in G0 state, conventional chemotherapy will be more efficient in the treatment of non-surgical hepatocellular carcinoma but further in vivo experiments on laboratory animals and analysis of absorbtion rate and side effects are required before we can go on and administrate arsenic trioxide to patients diagnosed with liver cancer.

Key words: Adjuvant chemotherapy, Cancer stem cells, Differentiation therapy, Hepatocellular carcinoma

Apoptosis Induced Pathways by RNA Interference VEGF Inhibition in Cervical Cancer

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Introduction:
Cervical cancer is a major cause of mortality in women, being considered one of the most aggressive types among gynecological cancers. This is due to the fact that these tumors are highly angiogenic, fact that enable the cells to invade the surrounding tissues. Literature describes vascular endothelial growth factor (VEGF) as a key molecule in triggering this process. Originally it was thought that the VEGF functions are mediated by paracrine mechanisms, but many tumors produce VEGF that can act as autocrine stimulators. Extensive studies have been done in VEGF targeted therapy but the mechanisms responsible for the anti-tumor activity are not yet fully understood. The aim of this study was to investigate if VEGF inhibition induces apoptosis in human cervical cancer HeLa cell line and the mechanisms by which this process is triggered.

Materials and methods:
VEGF gene inhibition was achieved by transfection of specific siRNA into HeLa stabilized cervical cancer cell line. As transfection agent we used a mixture of polilipides NeoFX SiPort from Ambion in OptiMem media. Quantification of VEGF gene expression after treatment was realized by real-time PCR at 24, 48 and 72 h after treatment. The apoptotic effect was monitored by chip flow cytometry. To identify the apoptosis induced mechanism of VEGF inhibition we analyzed the expression levels of 84 genes involved in the apoptotic process by PCR array technology.

Results and discussions:
Results showed a maximum inhibition for VEGF at 24 hours post treatment, the expression level of the VEGF gene being reduced by 70%. The maximum apoptotic effect was observed at 48h post transfection. PCR array analysis, showed deregulation of 6 genes at mRNA level, genes coding TNF receptor, IAP and BCL-2 family and genes involved in p53 and DNA damage patways.

Conclusions:
This study demonstrates that VEGF stimulates the cervix tumor cells by an autocrine mechanism and VEGF inhibition promotes apoptosis of the cells by both intrinsic and extrinsic pathways.

Key words: Cervical cancer, RNA interference, apoptosis, PCR array

p53 Gene Therapy in Human Cervical Cancer Cell Line

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Introduction:
Tumour suppressor gene, p53 can trigger cell death via apoptosis in many cell types, but the molecular mechanisms of which p53 induces apoptosis still remains to be fully understood. p53 can activate a variety of apoptotic and anti-apoptotic factors. The present study focused on evaluation of the role of p53, its target gene using p53siRNA, and their effects on mRNA gene expression based on PCR-array technology and HeLa cells as *in vitro* model.

Materials and methods:
HeLa cells (human epithelial cells, derived from cervical carcinoma) were reverse-transfected with 50 M p53siRNA using siPORT™NeoFX transfection agent. RNA was extracted using TRI®Reagent. RNA quality and quantity was measured with NanoDropND1000 and Bioanalyzer2100. Apoptosis gene expression levels were determined using Human Apoptosis RT^{2}Profiler™PCR-Array (SABiosciences) method.

Results:
The present study showed specific modulatory effects of p53siRNA as shown by the mRNA relative quantification. Our results revealed two genes statistically up-regulated ($p<0.05$), one from caspases family (Caspase-1) and one from TNF (tumour necrosis factor) ligand family (TNFSF10). In addition another ten genes, mainly with antiapoptotic role, were statistically down-regulated, from death domain and death effectors family, Bcl-2 family, CARD family and p53.

Discussions:
The experimental data suggests the restoration of TNFSF10 sensitivity and the activation of death receptors pathways. TNF ligands and receptors family are specific physiological mediators of apoptotic signalling, leading to the activation of proapoptotic signal and inhibition MAPK (mitogen activate protein kinases) and NFkB (nuclear factor-B) pathways, followed by caspase-1 activation.

Conclusions:
We have identified caspase-1 and TNSF10 as important target genes regulated by p53siRNA and it is expected to have an impact in anticancer therapy design. These findings provide new insights regarding the molecular mechanism, p53siRNA acts as a paracrine mediated-cell extrinsic celldeath response and can modulate the immune response and angiogenesis processes.

Key words: p53 gene therapy, RNA interference, HeLa cells, PCR array

Temozolomide Enhance Neoangiogenesis of Glioblastoma Multiforme in a 3D Angiogenesis Assay

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Introduction:
Glioblastoma multiforme (GM) is one of the most investigated tumor in relation with angiogenesis due to it's highly angiogenic property. Despite the combination of various treatments including surgery, radiotherapy and chemotherapy, patients diagnosed with GM have a poor prognosis. The aim of the study was to establish an in vitro three dimensional (3D) model that allows the evaluation of the functional aspects of tumor neoangiogenesis in GM and response to pro-angiogenic factors, anti-angiogenic drugs and Temozolomide (TMZ).

Material and methods:
Fresh tumor biopsies from patients with GM were grown in fibrin-gel matrix supplemented with serum-free growth medium. Pro-angiogenic (VEGF, b-FGF, EGF) and inhibitory factors (bevacizumab, doxorubicin) were added. The TMZ effects on tumor specimens were tested in different concentrations.

Results & Discussion:
We have found that GM has a strong vasculogenic potential in 3D angiogenesis model even after metronomic treatment with TMZ. In combination with bevacizumab TMZ could have an inhibitory effect on vessel formation. There is an interindividual variation which can explain the various responses that we have found. Further studies are required in order to better understand the mechanisms that are laying on TMZ resistance.

Conclusion:
Better selection of patients that are based on pre-treatment in vitro studies could improve treatment efficacy and long-term survival.

Key words: Glioblastoma multiforme, angiogenesis assay, temozolomide, in vitro model, Avastin

Placental Mesenchymal Stem Cells Differentiation

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Introduction:
Recent advances in studying stem cells have open new perspectives, by the use in cellular therapy. Current used sources are embryonic stem cells and adult stem cells, however these sources are raising a lot of problems, both ethical and technical. Thus, efforts were made to find other sources of stem cells which does not present ethical problems, are very easy accessible and in sufficient numbers for use in therapeutic purposes. The human placenta is a new source that fulfils all these conditions. Having

already at our disposal, stem cells from different compartments of placenta, already characterized from genetic and phenotypic point of view, we started to study the capacity of these cells to differentiate, especially to a hepatocytar and pancreatic line, with the idea of future transplantation to patients.

Material and method:

Stem cells populations (MAM, MAE and chorion) were cultivated in specific conditions for each type of cell trying to be obtained. Thus, complex differentiating protocols were used, that had more than one stage, in which the medium, the substratum and the growth factors had varied. The changes obtained at cellular level were evaluated after each stage, especially by determining specific markers gene expression for hepatic and pancreatic cells. For determining the level of differentiation and the moment of marker appearance, these markers were compared with those from completely differentiate cells. We also tried, to co-cultivate placental stem cells with completely differentiate cells obtained through biopsy from different organs and we evaluated the capacity of these cells to be induced with a certain phenotype. For use in cellular therapy it implies in vivo transplantation. To evaluate this possibility, we determined the presence of the major complex of histocompatibility (HLA). Our results confirmed anterior studies that report a weak or total absence of HLA gene expression, which is encouraging for the idea of using them on patiens. Also, we confirmed again the maternal phenotype of these cells.

Results and conclusions:

By traditional means of differentiation, we managed to induce partial hepatocytar phenotype with important morphological changes, to placental stem cells. In the case of pancreatic differentiation, by co-culture with cells obtained from pancreas though surgical interventions, we observed a transitory change in phenotype to exocrine component, and not to endocrine component.

Key words: Placental stem cells, hepatocytar phenotype, pancreatic cells, co-culture

A Static Cytometric Evaluation of the *Ex Vivo* Tumoral Chemosensitivity to Platinum-Based Drugs

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Introduction:

The platinum-based drugs have an important role in the therapeutic protocols of several tumor types. We developed a prediction marker of *ex vivo* chemosensitivity of tumor cells treated with platinum compounds, based on the new fluorescent cytometric technique.

Material and methods:

Primary tumor cell cultures were cultivated from 50 tumor fragments with origin in digestive, ovary, melanoma and other localizations. They were treated *ex vivo* with platinum-based chemotherapy agents: 12 cell cultures with cisplatin, 20 with carboplatin and 18 with oxaliplatin. After 24 hours of treatment cells were stained with fluorescent dyes flouresceindiacetate (FDA) and rhodamine Rh123 and they were placed on cell chips. Measurements were made using a laser scanning static cytometric system.

Results and discussion:

For every tumor cell culture, we analyzed both the platinum-treated and the untreated (control) cell populations. The static cytometric evaluation provides us fluorescence intensity (FI) and fluorescence

polarization (FP) values for every single cell positioned on the cell chip. The drugs inhibitory effect on tumor cell proliferation was assessed using FDA coloration, whilst the capacity of early apoptosis induction was evaluated with the Rh123 dye. Preclinical evidence suggests that increase of polarization (FP) values and decrease of intensity (FI) indicates a clear sensitivity to drugs. Increase in fluorescence polarization of FDA-stained cells was observed in 27 cell cultures, which is an evidence of the inhibitory effect induced by the chemotherapeutic agents. Moreover, among these cases, a confirmation of platinum compounds efficacy is the decrease in FI values in 21 tumors. Simultaneously with hyperpolarization and intensity drop, in 20 cases the measurements indicates an increase in Rh123 polarization values too, as a confirmation of early apoptotic processes.

Conclusions:

The polarization and intensity measurements provides us the mathematical parameters which contribute to metal compounds effect evaluation, and this conduct to optimization and personalization of the treatment and reduces the side effects of chemotherapy.

Key words: Platinum, cytometry, cancer, chemosensitivity

Functional Genomics Study in Prostate Cancer

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Introduction:

Prostate cancer represents a major health problem, being the second cause of death, after lung cancer, in the population of males over 50, worldwide.

Even the PSA blood test has changed the landscape of prostate cancer, testing for the early detection of prostate cancer remains a source of uncertainty and controversy. The application of DNA microarray technology to the study of prostate cancer will should improve knowledge of the genetic changes in the initiation and progression of this diseases.

Material and methods:

To identify differentially expressed genes that may be involved in prostate cancer progression, we compared normal tissues with prostate cancer tissues. Fifteen patients with prostate cancer, were included in the study. Normal tissues were collected from prostatectomy samples from five of these patients. Labeled complementary RNA (cRNA) from each tissue sample was hybridized to a pangenomic 44K 60-mer oligonucleotide microarray**.** We used Agilent oligonucleotide technology, one-color analysis, in which probes from tumor tissue and from the reference tissue are labeled by the incorporation of cyanine Cy3. Feature extraction software provided by Agilent was used to quantify the intensity of fluorescent images and to normalize results by subtracting local background fluorescence. All of the data were imported into GeneSpring GX software for database management, quality control, and analysis.

Results and discussion:

After normalization and correction of fluorescence intensities from the scanned images with Agilent Feature Extraction software, 36 379 statistically significantly regulated sequences were retained for analysis. We determined which genes were differentially expressed between the two groups. For this

analysis, we used a two-sample *t* test whose *P* values were adjusted by the false discovery rate step-up method of Benjamini & Hochberg to account for multiple testing. Our results show 1193 genes differentially expressed between two groups.

Conclusions:

Using microarray technologies we identified a cluster pf genes differentially expressed between normal and prostate cancer tissue. The genes are involved in cell cycle progression, transcription, angiogenesis, kinase activity.

Key words: Microarray, bioinformatics, prostate, markers

The Utility of Functional Genomics Technologies in Experimental Models for Cancer Research

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Cancer develops via a complex multistage cellular and molecular process. Each stage includes genetic and epigenetic events that progressively transform normal cells into highly malignant derivatives. The development of functional genomics technologies, like microarray and PCR array allows monitoring a large number of key cellular pathways simultaneously involved in cancer development. There is a desperate need to develop comprehensive analytical tools for studying heterogeneous disease like cancer. This need led to rapid evolvement of genomic and proteomic technologies, accelerating the rate and number of discoveries based on animal models. Combining genomics, proteomics and bioinformatics will lead to identification of new biomarkers and revolutionize the development of new drugs and therapies. Genetically modified mice with overexpressed and/or deleted genes have been used extensively as models for studying human cancer. However, it is uncertain if the mouse models reproduce the corresponding cancers in humans. Therefore, identification of mouse models that reproduce the molecular features of specific human cancers (or subclasses of specific human cancers) promises to accelerate both the understanding of the molecular pathogenesis of cancer and the discovery of new therapeutic targets. Establishing a molecular relationship between mouse and human cancers may also provide the basis for investigation the pharmacogenomics action of anticancer drugs. These discoveries based on animal models include understanding of cancer biology mechanisms as well as the identification of biomarkers supporting early detection, molecular classification of tumors, molecular predictors of metastasis, treatment response, and prognosis.

Key words: Animal models, genomics, bioinformatics

Monitoring T315I Mutation in Chronic Myeloid Leukemia by ARMS PCR

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Background:
The introduction of imatinib mesylate has revolutionized the treatment of chronic myeloid leukemia (CML). However, some cases develop resistance, due to point mutations.

Aims:
To assess the prevalence of the T315I mutation in a cohort of Romanian CML patients in order to adapt the treatment at any time the clinical and hematological aspects change.

Methods:
There were 41 CML patients (21 women, 20 men) with a median age of 51 years (24-85) diagnosed and treated in our institution between August 1996 and December 2008. At the moment of sampling, 34 patients were in chronic phase, 2 patients in accelerated phase and 5 patients in blastic phase. 32 patients (28 in chronic phase, 3 in accelerated phase and 1 in blastic phase) had been treated with tyrosine kinase inhibitors (TKI), for variable intervals (1-60 months, median 21). 32 patients had received imatinib, 400-600 mg/day (12 as first line treatment, 20 as second and third line) and 3 patients had received dasatinib (2 as second line and one as third line treatment). The T315I mutation was studied by an ARMS PCR (Amplification Refractory Mutation System - Polymerase Chain Reaction) technique.

Results:
At the moment of sampling 26 of the 32 patients treated with TKIs (81.2%) were in complete hematologic remission (CHR), 15 patients (46.8%) had a complete cytogenetic response (CCyR), 4 patients (12.5%) had a partial cytogenetic response (PCyR) and 9 patients (28%) had a minor cytogenetic response (mCyR). 3 patients (9.3%) had a major molecular response (MMR). 2 of the MMR patients were treated with imatinib and one with dasatinib. The T315I mutation was found in only one patient, diagnosed in 1998 who was in clinical and hematological relapse and in accelerated phase after several lines of treatment (interferon + Ara-C, imatinib, dasatinib).

Summary/conclusions:
The T315I mutation was a very rare event in our patient population, despite a high proportion of patients who had a suboptimal response after TKI treatment. An explanation could be the fact that few of our patients were treated as first line therapy with TKIs; also, few patients were treated with high-dose TKIs. Further follow-up of the patients treated as first line-therapy TKI may result in a higher rate of T315I detection. This aspect is of utmost importance as such CML patients may be the ones who will actually benefit from allogeneic stem cell transplantation.

Key words: Chronic myeloid leukemia, mutation, tyrosine kinase

The *JAK2* rs10974944 SNP, Part of *JAK2* 46/1 Haplotype, is Strongly Associated with *JAK2* V617F – Positive Myeloproliferative Neoplasms

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Introduction:
Polycythemia vera, essential thrombocythemia and myeloid metaplasia with myelofibrosis are myeloproliferative neoplasms, characterized in a majority of cases by a unique somatic point mutation, *JAK2* V617F. Very recently, it was shown that the *JAK2* V617F mutation would occur more frequently on a specific *JAK2* haplotype, named *JAK2* 46/1.

Material and methods:
We genotyped 149 myeloproliferative neoplasms patients (69 had polycythemia vera, 65 had essential thrombocythemia and 15 had myeloid metaplasia with myelofibrosis) with a known *JAK2* V617F mutational status and 150 healthy controls for the *JAK2* rs10974944 (C/G) SNP (single nucleotide polymorphism), in which the G allele tags the 46/1 haplotype.

Results:
We found that GG/CG genotypes were significantly enriched in patients compared to controls ($p < 0.0001$). After stratifying for the *JAK2* V617F mutational status and for the mutant allele burden, we demonstrated that GG/CG genotypes were significantly more frequent in V617F positive compared to V617F negative patients ($p = 0.001$), but not in V617F negative patients compared to controls ($p = 0.29$). Similarly, the GG/CG genotypes were significantly enriched in V617F positive patients with a mutant allele burden >50% compared to those with a mutant allele burden <50% ($p = 0.0006$).

Discussion:
Our results indicate that the G allele of the *JAK2* rs10974944 SNP, part of the *JAK2* 46/1 haplotype, contributes significantly to the occurrence of *JAK2* V617F - positive myeloproliferative neoplasms. Moreover, *JAK2* 46/1 seems to be associated with mutant allele burden >50% in *JAK2* V617F - positive myeloproliferative neoplasms patients.

Conclusion:
By an unknown mechanism, the *JAK2* 46/1 haplotype seems to explain in part the acquisition of the somatic mutation *JAK2* V617F. In the future, other constitutional variants involved in the etiopathogenesis of the myeloproliferative neoplasms will be probably identified.

Key words: myeloproliferative neoplasms, *JAK2* V617F, *JAK2* 46/1 haplotype, *JAK2* rs10974944 SNP

DNA Methylation in Cancer

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Cancer is caused by inappropriate function of genes that promote or inhibit cell growth or survival can be caused by errors introduced into the genetic code itself or by faulty epigenetic mechanisms deciding which genes can and cannot be expressed. Genomic DNA methylation is one of the most important epigenetic modifications in eukaryotes. DNA methylation patterns undergo complex changes in cancer.

Studies have indicated that epigenetic changes might 'addict' cancer cells to altered signal-transduction pathways during the early stages of tumor development. Dependence on these pathways for cell proliferation or survival allows them to acquire genetic mutations in the same pathways, providing the cell with selective advantages that promote tumor progression.

Breast cancer is among the most frequently diagnosed neoplasias and the second leading cause of cancer death amoung women. Its' initiation and progression is associated with aberrant DNA methylation and expression of genes that control: the epithelial-mesenchymal transition (EMT), a critical step in malignant conversion, the antitumor immune response by T lymphocytes (*TNFSF7*) and cell differentiation and maintenance of cellular homeostasis (*beclin 1*).

Because hormone receptor (*ER* and *PR*) and *HER-2* status can determine prognosis and response to therapy in breast tumors, various researches focused on correlating methylation of several tummor suppressor genes with the expression of ERs and PRs. A recent study showed that hypermethylation of estrogen receptor α (*ERα*) might cause hyperactivation of cellular kinase signaling, notably of Akt, described as a selective survival advantage for primary tumor cells even in the presence of anti-estrogens.

Futhermore, recent data suggest that estrogen-mediated suppresion of PTPRO is probably one of the early events in estrogen-induced tumorigenesis.

The advances in comprehension of DNA methylation patterns that modulate chromatin conformation, gene transcription and diverse cellular signaling pathways have provided the essential basis for the current development of epigenetics-based biomarkers and drugs for the diagnosis and treatment of breast cancer.

Key words: DNA methylation, breast cancer, hormone receptor, signaling pathway

Sporadic Colorectal Cancer or Hereditary Lynch Syndrome: Anamnestic Epidemiologic Study Using Amsterdam and Bethesda Criteria

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Introduction:
About recently, CRC revealed to be the most prevalent digestive neoplasia in Romania. There exist no data on LS in our area.

Objective:
The aim of our retrospective study was to appreciate the sporadic or hereditary nature of colorectal cancers identified in our department, according to Amsterdam II and Bethesda revised clinical criteria.

Methods:
Our study included 96 patients with newly diagnosed CRC (March 2006 - March 2009). Colonoscopy and biopsy were used for the diagnosis and CT for staging. Family history of cancer was identified by anamnesis. The following aspects were analyzed: percentage of newly diagnosed CRC as compared to other digestive cancers, age of the patients, localization of CRC (left/right colon), family history of cancer (CRC/extra colonic), and number of generations involved.

Results:
CRC was recorded in 96/252 (38%) of the total number of patients with digestive cancers diagnosed in the same period. CRC appeared to be sporadic in 78/96 (81.2%). Anamnesis revealed a family history of cancer in 18/96 (18.8%) of patients, site-specific (CRC) in 6/18 (6.3%) and extra colonic cancers in 12/18 (12.5%). The localization of CRC, left/right colon, was of 1/1 in patients with positive family history of cancer and of 3.8/1 in patients with negative history. One patient fulfilled the Amsterdam criteria for LS. Thirteen patients fulfilled the Bethesda criteria for LS.

Conclusion:
In our population, CRC appeared to be sporadic in most patients, 81,2%. According to Amsterdam II and Bethesda revised criteria, 14 patients should have their tumors be tested for MMR gene mutations, as molecular screening tests for LS.

Key words: Colorectal cancer, hereditary Lynch syndrome, epidemiology

How to Boost the Breast Tumor Bed? From Conventional to 3D-Conformal in Early Breast Cancer Radiotherapy in "Prof. Dr. Ion Chiricuță" Oncology Institute Radiotherapy Department

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In breast cancer, radiotherapy is given after conservative or radical surgery, adapted to patient and tumor characteristics. The main goal of radiotherapy is to eradicate residual tumor deposits and to reduce their potential risk of dissemination. Radiotherapy given either after conservative surgery to N0/N+ patients (50-60 Gy) or after radical surgery to node positive (N+) patients (40 – 50 Gy), does not just reduce the local recurrences risk (LRR), but also improves long term survival. The local recurrences rate is significantly reduced by boosting the tumor bed with 10-16 Gy. The absolute reduction in the local recurrence rate as a result of the boost is 10 times greater for younger patients. 3D planning based on a CT-scan of the breast is necessary. CT simulation offers the potential to move to a 3D planning "zone", where clinical target volume and organs at risk can be defined and margins can be added to account for the organs movement or set-up errors. All geometric uncertainties will be incorporated into the breast planning process, and, finally, the field's geometry can be established.

Key words: breast cancer, radiotherapy, quality assurance

Laparoscopy – Modern Treatment in Cervical Cancer

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Cervical cancer is a serious chronic illness, major medical and social developments with very severe, especially when it is detected in advanced stages. Importance of the problem stems from the fact that cervical neoplasm is a leading cause of death of the female population, ranking second in frequency, being placed first breast cancer. Laparoscopic surgical techniques can be used in stages 0, I and II. In stage IIB and III laparoscopic surgery will be integrated into the radio complex surgical treatment. Laparoscopic surgery was required because of its benefits as: reduction of postoperative pain, short hospitalization, shorter postoperative recovery with return to normal life soon. The study: prospective and retrospective consists of a total of 74 cases aged 34-68 years who received surgery after a diagnosis of certainty represented by histopathology. Patients admitted in the University Clinic of Obstetrics and Gynecology "Bega", Timișoara, between 01.01.2009-31.12.2009 were diagnosed with cervical cancer. Depending on the distribution of cases lesional stages (FIGO staging) cases we observed the following distribution: stage IA - 3 cases, stage IB - 7 cases, stage IIA - 18 cases. Only these 28 cases, the intervention was practicing laparoscopic hysterectomy with evidare radiacala node. The average number of lymph nodes removed was 20, and the limits of resection were free of cancer in all cases.

Conclusion:

Laparoscopically assisted vaginal radical hysterectomy is a safe and effective in the treatment of cervical cancer in the stadium early.

Key words: Cervical cancer, laparoscopic surgery, hysterectomy

Philadelphia Positive Acute Lymphoblastic Leukemia. The Experience of Hematology Clinic Cluj-Napoca

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Background:
The prognosis of Philadelphia chromosome (Ph) positive acute lymphoblastic leukemia (ALL) is poor. But the incorporation into frontline treatment of ABL kinase inhibitor imatinib has significantly improved the antileukemic efficacy of induction therapy and the prognosis in these patients.

Aim:
To assess the prevalence of Ph+ ALL in a cohort of Romanian ALL patients and their outcome (under treatment with tyrosine kinase inhibitors and chemotherapy).

Methods:
There were 35 patients diagnosed with ALL and treated in our institution between 2007 and 2009. There were 20 males (57%) and 15 females (43%), with median age of 38 (19-66). 15 patients were diagnosed with preB/common ALL, 7 patients with mature B cell ALL, 11 patients with T cell ALL and the phenotype could not be determined in 2 patients. Of all patients, 8 presented Ph+ALL. 4 patients presented complex karyotype (1 associated with Ph chromosome), there was a duplication of Ph chromosome in 1 patient and a loss of chromosome 9 in 1 patient. Among the Ph+ALL patients the major breakpoint cluster region (BCR) rearrangement predominated and was found in 5 patients, while the minor BCR was determined in 3 patients. 24 patients presented with leucocytes<30000/µl and 11 patients with >30000 leucocytes/µl. There were 3 patients with clinical CNS disease and 2 other patients with blastic meningitis with no clinical expression. Imatinib was administered in 6 out of 8 Ph+ALL patients, 600 mg/day associated to chemotherapy.

Results:
We observed 25 complete hematological responses. There were 10 patients with no response to therapy. Of the 8 Ph+ALL only 2 patients did not obtain complete hematological response, 1 patient obtained complete cytogenetic response and 1 patient major molecular response. There were 11 relapses among the whole group, only one in the Ph+ALL group. The median overall survival (OS) (determined by Kaplan Meyer) for our cohort was 36 months and the median disease free survival (DFS) was 24 months. The median OS for Ph+ALL patients was not reached, 53% are still alive. The median OS for non Ph+ ALL patients is 36 months. The median DFS for Ph+ALL patients was not reached, 53% are in remission and the median DFS for nonPh+ ALL patients is 26 months.

Conclusions:
Although OS and DFS seem better for Ph+ALL patients the differences are not statistically significant.

Key words: Acute lymphoblastic leukemia, Ph chromosome, tyrosine kinase inhibitor

Acute Myeloid Leukemia and Type II Diabetes Mellitus in Complete Remission after Allogeneic Stem Cell Transplantation

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Background:
Bone marrow transplantation is becoming an important part of the treatment of hematologic disorders, but also of metabolic disorders and autoimmune diseases. Using animal models for autoimmune diseases it has previously been demonstrated that allogeneic bone marrow transplantation can be used to treat autoimmune diseases such as insulin-dependent diabetes mellitus.

Aims:
To present the case of a patient diagnosed with acute myeloid leukemia (AML) and type II diabetes mellitus, both in remission after allogeneic stem cell transplantation.

Method:
AS is an AML patient, 54 years old, diagnosed in our institution with AML- M1, normal kariotype (FLT3 and NPM1 wild type) in January 2008. Between January and July 2008 she was treated with 3+7+Etoposide induction chemotherapy (according to a German protocol?) and 4 courses of high dose Ara-C and Idarubicine chemotherapy. The patient had previously been diagnosed with type II Diabetes Mellitus and she was under insulin treatment maintained during the entire chemotherapy. In October 2008 the patient underwent allogeneic stem cell transplantation,with Busulfan+ Cyclofosfamide conditioning, from the patient's brother. And ever since the patient is under immunosuppressive treatment with cyclosporine.

Results:
The complete hematological response was obtained after the induction chemotherapy and maintained ever since. After the stem cell transplantation the hematological recovery is satisfactory with the persistence of mild thrombocytopenia and anemia; and mild lymphocytosis. The chimerism is 99%. Immediately after the transplantation was done there was no need for insulin treatment, since the blood glucose was constantly under 150 mg/dl. There was an episode of acute GVHD of hepatic and cutaneous involvement which resolved with corticosteroid therapy and an increase in cyclosporine administration. No rise of serum glucose was observed despite the corticosteroid therapy. Currently there is a grad I chronic GVHD of the liver.

Conclusions:
Our patient's case is a fortunate one where remission of both hematologic disorder and autoimmune disease was obtained by allogeneic stem cell transplantation. It is possible that in the near future allogeneic stem cell transplantation will become a powerful strategy for treating patients with hematologic disorders and various intractable diseases, including autoimmune diseases.

Key words: Acute myeloid leukemia, diabetes mellitus, allogeneic stem cell transplantation

Risk Factors for Ineffective Coping in Acute Leukemia Patients

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Introduction:
Coping with a diagnosis of cancer, in general, and acute leukemia, in particular represents a dynamic process influenced by medical, psychological and social issues. Many psychooncologists emphasize that some patients adapt better than do others ant that psychosocial support services cannnot yet be offered to every patient. For this reason it is important to determine wich patient is at greatest risk of developing adjustment problems in order to offer them psychological support.

Material and methods:
We have done 100 interviews taken from acute leukemia patients in various disease stages and various therapeutically steps treated in the Hematology Clinic in Cluj-Napoca during November 2007 – May 2009.Patient interviews were conducted using a questionnaire divided in an introductory part (consisting in informed consent and explaining the reason of the intervew) and 10 specific sections. The following topics were pursued: analysis of the way the diagnosis was communicated and the patient`s psychological reaction to diagnosis; the five-stage process of coping with the malignant diagnosis (denial, anger, bargaining, depression, acceptance); identification of factors with a facilitating effect in overcoming an ineffective coping and developing an effective coping; interval from diagnosis to acceptance; the level of acceptance; evaluation of patient internal and external resources; understanding different types of coping with malignant disease symptoms and therapy side effects; coping with substitution therapy with blood and blood products; coping with death of a fellow patient; taking self-blame for the disease; disease effect on patient's life at personal, family, professional and social level; family behavior regarding the disease; self-assessment of coping. Based on the interviews, we evaluate the efficiency of coping (from a psychosocial counselor's and a haematologist's perspective) and the statistical significance of correlations between coping and 25 other variables (such as patient personality traits, patient`s demographic characteristics, disease-related and treatment-related issues, diagnosis communication, diagnosis acceptance, time passed to diagnosis acceptance, isolation in hospital, patient`s psychological resources, selfblaming for the disease and others). Data was analyzed using SPSS. Statistical significance for various qualitative variables association was evaluated using the Pearson's χ^2 test and Fisher Freeman-Halton's exact test in cases when at least 20% of the expected count less than 5. The study has the agreement of Ethic Committee of "Iuliu Haţieganu" Medicine and Pharmacy University Cluj-Napoca, Romania.

Results:
Risk factors for inefficient coping in acute leukemia patients are:- Lack of sense of humor (OR=12.6, 95% CI: 1.45-109.39)- Lack of fighting spirit (OR=23.33, 95% CI: 3.94-138.098) Inefficient coping in acute leukemia patients is statistically significant associated with:- Lack of sense of humor($p=0.008<0.05$, statistically significant)- Lack of fighting spirit($p=0.0005<0.05$, statistically significant)- Hopelessness/ helplessness ($p=0.00031<0.05$, statistically significant)-Low acceptance of diagnosis, i.e. values between 4 and 6 on a 1 to 10 scale ($F=7.13$, $p=0.019<0.05$, statistically significant)

Conclusion:
Analising 25 factors, only lack of humor and lack of fighting spirit representing risk factors for inefficient coping, so that these patient should benefit of targeted and individualised psychological support.

Key words: Ineffective coping, risk factor, acute leukemia

The Treatment with Bortezomib in Multiple Myeloma

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Major advances have occurred in the understanding of the biology and the tratment of multyple myeloma in the past decade.Most importantly, new agents as Thalidomide, Lenalidomide and Bortezomib have emerged as new active agents and are being icorporated rapidly into the treatment of both newly diagnosed and refractory multyple myeloma.

Aim:

The aim of this study was to evaluate the response to Bortezomib with Dexamethazone in patients with refractory multiple myeloma treated in the haematological departement of "Prof. Dr. Ion Chiricuță" Oncology Institute from Cluj-Napoca.

Patients and methods:

22 patients with refractory multiple myeloma were included: 12 males, 10 females, median age 59 years (51-67). 10 was IgG, 8 IgA, and 4 only light chain, 84% had osteolysis. According Salmon and Durie staging system all the patients were in the III stage, 14 patients in the stage IIIA and 8 patients in the stage IIIB. Bortezomib was administrated in the dose of 1,3 mg/kg on days 1, 4, 8, 11 in a 21 day cycle for 8 cycles with Dexamethazone 40 mg days 1-4 of each cycle.

Results:

Response rate according to the IMWG criteria for the patients who receved Bortezomib with Dexametazone was 31,8% (0,99%nCR and 30,91%PR) Median time to progression for responders was 6 month.

Conclusion:

Bortezomib is an efficient drug in refractory multiple myeloma, with a response rate of 30-31%.

Key words: Bortezomib, multiple myeloma, Salmon and Durie staging system, Response rate

Unusually Long Lasting Hematological Remission and Survival in a Case of Chronic Myeloid Leukemia Treated with Three Different Thyrosine Kinase Inhibitors

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Background:

The natural history of chronic myeloid leukemia (CML) is characterized by a biphasic and sometimes triphasic course. The overall prognosis for patients in blastic phase (BP) is poor. Approximately 50% of patients have a myeloid phenotype, 25% have a lymphoid phenotype, and 25% have an undifferentiated phenotype. In myeloid BP, AML induction regimens are associated with overall response rates of 20 to 30%. Most responses are transient with a median survival of 4 to 10 months. Imatinib mesylate (Glivec) is a specific inhibitor of BCR-ABL tyrosine kinase. In patients who have newly diagnosed chronic phase

CML, Glivec induces 95% complete hematological, 80% complete cytogenetic and 25% complete molecular response. In myeloid BP, the response rate is 55% but the responses are usually short (mean 10 months).

Case report

Patient SM, male, 29, was diagnosed with Ph[1] positive CML in August 1996. He was initially treated with hydroxiurea and interferon. In November 2000, the patient is admitted for fever, bone pain, marked splenomegaly (20 cm under the costal margin). The WBC was 226,000/μl with 32% blasts in peripheral blood and 34% in the bone marrow. Various combination chemotherapy regimens were given (high dose hydroxiureea with Ara-C, etoposide, thioguanine) without a significant response. In august 2001 Glivec at 400mg/day was initiated. At the time, the WBC was 69,000/μl with 28% blasts and the splenomegaly was 20cm under the costal margin. A complete clinical and hematological response was obtained in only 13 days. Since July 2004, due to increased leucocytosis, hydroxiurea was added. Under this combination the patient was non-symptomatic, with WBC below 10,000/μl, myeloblasts 1-2% and promyelocytes 2-4%, until January 2009. The patient was started on dasatinib 100mg/dl in February 2009 and is in complete hematological remission since then. Starting from October 2009 – until now the patient received Nilotinib 400 mg/day. Currently he is in a good hematological response with 10 – 20 000 WBC and 1 – 2% myeloblasts. From 2001 until now the periodical cytogenetical examinations showe a lack of cytogenetic response, with 95-100 Ph[1] positive mitoses.

Conclusion:

Tyrosine kinase inhibitors (TKI) represent a viable alternative in blastic phase CML. To our knowledge, this case displays the longest reported survival of a patient in blastic phase CML treated with tyrosine kinase inhibitors.

Key words: Chronic myeloid leukemia, Imatinib mesylate, Dasatinib, Nilotinib

Mabthera in Treatment of Nonhodgkin Lymphomas- Presentation of 27 Cases Treated at Hematology Clinics Cluj-Napoca between 2001-2009

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Anti- CD20 monoclonal antibodies (Mabthera or Rituximab) were introduced in the last few years as new treatment options in nonhodgkin lymphoma (NHL). Using of Mabthera as single agent in indolent lymphomas or in combination with chemotherapy in aggressive lymphomas led to significant improvement in remission rate,disease free survival and long-term survival. Recently, Mabthera was introduced as maintenance therapy , raising even more the chances of cure. We analysed 27 cases of NHL treated with Mabthera in our hospital. There were 18 women (66,66%) and 9 males (33,33%). Age of patients was between 19 and 75 years, with an average of 45 years.Most of them were diffuse large B cell NHL, the remainder being : follicular, lymphocytic, mantle cell and marginal zone lymhpomas. The majority of cases were diagnosed in advanced stage – III and IV according to Ann_Arbor staging. We applied the international scoring system (IPI and FLIPI) to all cases, according to that there were 11 cases with low risk (40,75%), 21 with intermediate risk (55,5%) and 1 case with high risk (3,75%). Mabthera was applied as first line therapy to 16 cases (60%), the rest (40%) received Mabthera as salvage therapy.Only 2 cases received Mabthera as single agent, while the majority of pacients were

treated with combination therapy.Complete remission was obtained in 18 pacients (66,66%), partial remission in 3 cases (11,1%) and 6 patients (22,3%) were refractory. Most of the refractory patients were those who received this treatment as second line option.Although our number of cases is small in compared to other countries, our data are concordant to that reported in literature.

Key words: Mabthera, Non Hodgkin Lymphoma

Unusual Alternative of Richter Syndrom: Hodgkin Lymphoma

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Generally, the term of Richter syndrome concerns a non-Hodgkin lymphoma with large cells, aggressive which appears as particularly evolution to 3-10% patients with chronic lymphatic leukaemia with B cells. As a general rule these cases appear as advanced stages Ann-Arbor with a poor treatment response. A 55 years old male whose diagnosis of chronic lymphocytic leukaemia with B cells was established on January 1998, clinical appears to have generalized lymphadenophathy of 2-4 cm and hepatosplenomegaly. Haematologically : leococitosis with 97% lymphocytes, thrombocytopenia. It follows an immediate treatment with chlorambucil. Since September 2001 the patient complains heavy perspirations during night, hepatosplenomegaly is more evident and appear abdominal mass nodules. On June 2002 as a result of axilar lymphadenopathy biopsy, a chronic lymphocytic leukaemia is established. All these generally aspects are a motive for a new biopsy. After hystopathological examination in conjunction with immunocytochemical staining is establishing the diagnosis of Hodgkin lymphoma of lymphocyte predominant type. The patient was treated with three cycles of VCAEP but without response. After 5 months since the diagnosis was established the patient dies of septicaemia. It is a Hodgkin lymphoma as a seldom alternative of Richter syndrome. This evolution with its generally marks and lack of response of cytostatic treatment represents the progressive evolution of chronic lymphocytic leukaemia.

Key words: Chronic lymphatic leukaemia, Richter syndrome, Hodgkin lymphom

Developing a Predictive Model for Transfusion Requirement for Acute Leukemia Patients

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Introduction:
Faced with the ongoing reduction in the number of blood donors, the clinical hematologist must often ask patient families for help in finding suitable donors. Another benefit of the predictive model

is improved communication with the blood bank, allowing the clinical hematologist to order the appropriate amount of blood units necessary for the whole duration of the aplasia.

Material and Methods:

Study type: analytical, cohort, retrospective. We studied 246 patients with acute leukemia admitted in the Hematology Clinic in Cluj-Napoca during 1995-2008 and treated according to international protocols (exclusion of palliative care therapy, deceased or transferred patients). 860 aplasia episodes secondary to chemotherapy were included in the study. All the patients signed the informed consent for the blood transfusions. The study has the agreement of Ethic Committee of "Iuliu Haţieganu" Medicine and Pharmacy University Cluj-Napoca, Romania. Statistical analysis was performed using a linear model (multiple regression).Independent variables used in setting out the predictive models were: age, gender, morphologic type of leukemia, bone marrow blast infiltrate, disease stage at the beginning of chemotherapy regimen (diagnosis, complete remission, partial remission, refractory disease, no response), chemotherapy regimen number, medullar erythrocyte line (normal, low, dysplastic), chemotherapy type (high-dose or standard-dose), hemoglobin levels at chemotherapy start and at the end of the aplastic period, hemorrhagic episodes during the next aplastic period, hemolytic transfusion reaction, compatibility testing type for transfused blood (classic or gel migration methods). Finding the best regression model was based on univariate analysis of all risk factors (chi-square tests). Multicollinearity was excluded when heavily correlated pairs of independent variables were found. Statistical analysis was performed using SPSS, Statistica and Excel.

Results:

The number of blood units necessary to be transfused to an acute leukemia patient in an aplastic phase following chemotherapy can be estimated using the following equation: ***Number of blood units = 7.01 – 0.35 x Starting Hb + 0.77 x Diagnosis – 0.91 x Partial remission – 1.01 x Complete remission + 1.21 x Minor hemorrhages + 3.41 x Major hemorrhages + 1.31 x Haemolysis.*** "Number of blood units" is the amount of whole blood and/or red blood cells concentrate units necessary. Independent variables "Diagnosis", "Partial remission" and "Complete remission" (disease stage at chemotherapy), "Minor hemorrhages" and "Major hemorrhages" are assigned 1 if present and 0 if absent. Minor hemorrhages were represented by low quantity epistaxis, bleeding gums, purpura, etc. Major hemorrhages were represented by menorrhagia, haematemesis, melena, etc. This model has statistical significance (ANOVA test, $p<0.05$, statistically significant) therefore it can be generalized from the study group to the whole population.

Conclusions:

The variables representing the best predictors of blood units number transfused to an acute leukemia patient in an aplastic phase following chemotherapy were: hemoglobin levels at chemotherapy start, disease stage at the beginning of chemotherapy regimen, hemorrhagic episodes during the next aplastic period and hemolytic transfusion reaction.

Key words: Predictive model, transfusion requirement, acute leukemia

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