## CELL KINETICS CONTRIBUTIONS TO THE DIFFERENTIAL GENETIC PATTERN OF C-CELL AND ADRENAL MEDULLARY HYPERPLASIAS IN MULTIPLE ENDOCRINE NEOPLASIA 2A

M. de Miguel,[1] A. Blanes,[2] R. Tashjian,[3] S.J. Diaz-Cano,[3,4]
University Hospitals ([1]Seville and [2]Malaga, Spain), [3]Tufts University (Boston, MA), and [4]Barts and The London School of Medicine (London, UK),

**Introduction**: Cell kinetic contributions to the genetic heterogeneity of C-cell hyperplasias (CCH) and adrenal medullary hyperplasias (AMH) in multiple endocrine neoplasia 2A remain unknown.
**Methods**: We selected 22 CCH foci and 34 AMH nodules from MEN-2A kindred (*RET* mutation in codon 634). DNA extracted from microdissected samples was used for microsatellite analysis of *TP53*, *RB1*, *WT1*, and *NF1* by PCR-denaturing gradient gel electrophoresis. Ki-67, in situ end labeling (ISEL), and kinetic (Ki-67/ISEL) indices were calculated in each sample. Only informative cases were included.
**Results**: CCH revealed higher and more homogeneous incidence of loss of heterozygosity (LOH) for [*TP53* (12/20, 60%), and *RB1* (8/14, 57%)] than AMH [*TP53* (9/31, 29%), *RB1* (3/25, 12%), *WT1* (9/28, 32%), and *NF1* (9/19, 47%)]. Coexistent LOH in ‡2 loci were observed in 5/20 CCH and 6/31 AMH, always involving *TP53* in CCH and *NF1* in AMH. Kinetic indices were significantly higher in lesions accumulating multiple LOH than in lesions with single LOH (261.25 vs. 169.38), due to decreased ISEL indices.
**Conclusions**: MEN 2A lesions accumulating somatic genetic abnormalities reveal the most advantageous kinetic profile, essentially due to down-regulated apoptosis. Differential genetic profile contributes to that pattern, involving *TP53/RB1* in CCH and *NF1* in AMH.

## Systemically transplanted bone marrow gives rise to osteoblasts and osteocytes *in vivo*

S Omorphos, J A Hoyland, C L Le Maitre, J A Liu Yin*, G S Lucas*, R J Byers
Laboratory Medicine Academic Group & * University Department of Haematology, University of Manchester, Oxford Road, Manchester, M13 9PT

Animal studies have demonstrated that infused bone marrow derived mesenchymal progenitor cells (BMMPC) have the ability to circulate and home into several tissues of the recipient animal, giving rise to the tissue in which they reside. However, the transplantability of BMMPC in humans is still a controversial issue.

This study investigated the engraftment capacity of BMMPC in 7 female patients who had received sex-mismatched conventional bone marrow transplants. A fluorescent in-situ hybridisation (FISH) technique was used to detect the Y-chromosome in bone marrow trephines of the sex-mismatched cases. The Y-chromosome was detected in osteoblasts and osteocytes, identified by their position in the bone, indicating that they are derived from donor BMMPC, in 6 out of the 7 patients. No hybridisation signal was detected in any of the negative controls. Statistical analysis showed that donor BMMPC have a bone marrow homing efficiency in the range of 0.00%-2.96%.

To our knowledge, this is the first successful transplantation study performed using bone marrow trephines. Our results indicate the ability of BMMPC to transit within the peripheral circulation in humans, reside in the bone marrow and give rise to bone. This study opens the door for the future therapeutic use of BMMPC in humans, in particular for bone defects and post-menopausal or age-related osteoporosis.

## Leptin and leptin receptor expression in human bone

Rinaldi S, Hoyland JA, Braidman IP
Laboratory Medicine Academic Group, University of Manchester, Manchester, UK

Central administration of leptin reduces bone mass, but its addition to cultures of the appropriate precursor cells promotes osteoblastogenesis and inhibits osteoclastogenesis. However, the identity of leptin (ob) and leptin receptor (ob-r) expressing cells in human bone *in vivo* remains unclear. We therefore investigated leptin and leptin receptor localisation and mRNA expression in human bone by indirect immunoperoxidase and RT-PCR respectively.

Polyclonal antibodies were used to the carboxy termini of ob and ob-r, raised in rabbits and goats respectively. Pressure cooker retrieval was necessary for antigen detection. In sections of breast carcinoma, used as positive control, cytoplasmic immunoreactivity was seen in ductal epithelial and tumour cells with both antibodies. In decalcified paraffin sections of fracture callus, normal, hyperparathyroid, Pagetic, and osteoporotic bone leptin was localised to the cytoplasm of osteoblasts and their precursors, proliferating chondrocytes, newly incorporated osteocytes, osteoclasts, and some marrow stromal cells. Ob-r immunoreactivity was similar, except that osteoclasts were negative.

With RT-PCR, using RNA extracted from normal and osteoporotic bone, transcripts for the common and signalling portions of the leptin receptor were universally observed. Leptin mRNA was also detected in osteoporotic and normal samples.

These observations support the hypothesis that leptin may have an autocrine or paracrine action in bone.

## Immunohistochemical Detection of CD117 Expression in Soft Tissue Sarcomas

M Sabah, R Cummins, E Kay and M Leader
Department of Histopathology, Beaumont Hospital and Royal College of Surgeons in Ireland, Dublin

C-kit proto-oncogene product (KIT, CD117) is a tyrosine kinase receptor for stem cell factor. It is expressed in a variety of normal cells, including haematopoietic stem cells, mast cells, melanocytes, germ cells and interstitial cells of Cajal. CD117 expression is used as a marker for discriminating gastrointestinal stromal tumours (GISTs) from other sarcomas. In this study CD117 expression in a wide range of soft tissue sarcomas was examined.
Methods and results: A total of one hundred and fifty five cases of different types of sarcomas were evaluated by immunohistochemical staining with the CD117 antibody. Positive staining in more than
80 % of the tumour cells was present in 41 % of all the cases examined and was a consistent feature of GISTs and synovial sarcomas. Focal staining in neoplastic cells accounting for 30% or less of the tumour was identified in 18% of all cases examined.
Conclusion: CD117 is not specific to GISTs, but is also expressed in a wide variety of sarcomas. Awareness of this finding is of diagnostic importance since many sarcomas may involve the intestinal wall and simulate GISTs. Furthermore, tyrosine kinase inhibitor, STI 571, is currently being evaluated in the treatment of patients with GISTs. Whether this has any therapeutic role in other neoplasms expressing Kit protein remains to be elucidated.

# Cell kinetics contributions to the differential genetic pattern of C-cell and adrenal medullary hyperplasias in multiple endocrine neoplasia 2A

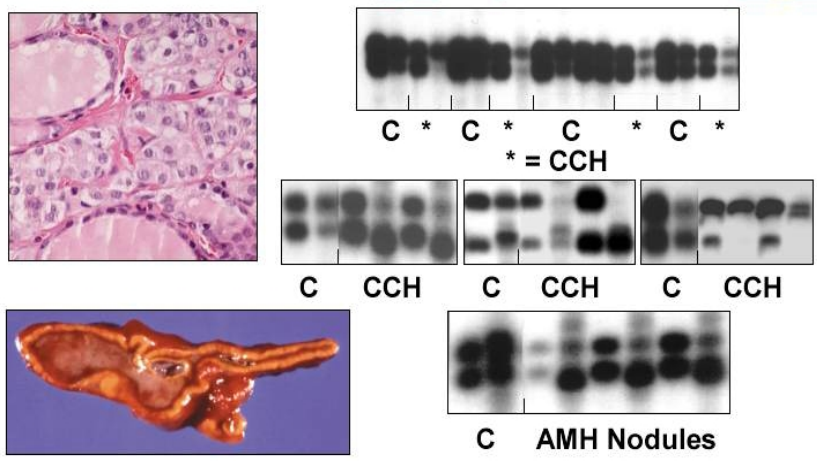
*M de Miguel, A Blanes, R Tashjian, S J Diaz-Cano*

*The Journal of Pathology. 01/2002; 198:12A.*
*DOI: 10.1002/path.1711980102*

## Proliferative Lesions in MEN 2A

- Histologic definitions of C-Cell Hyperplasia (CCH) and Adrenal Medullary Hyperplasia (AMH) are controversial
- They show a multifocal growth patterns and heterogeneous genetic profile
- The contribution of cell kinetic to the genetic pattern remains unknown

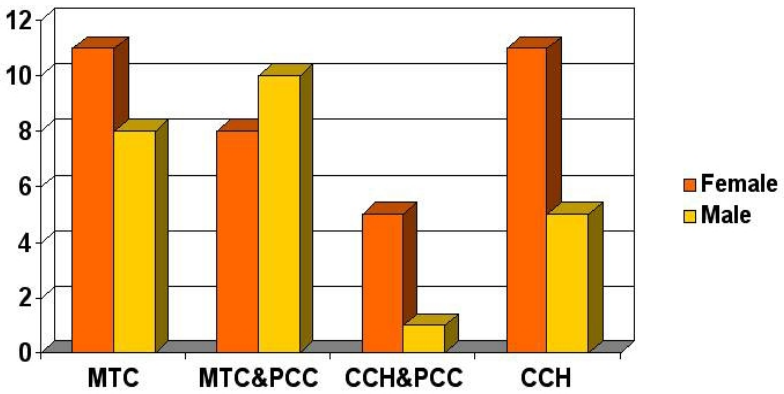
## HUMARA Patterns in MEN 2A



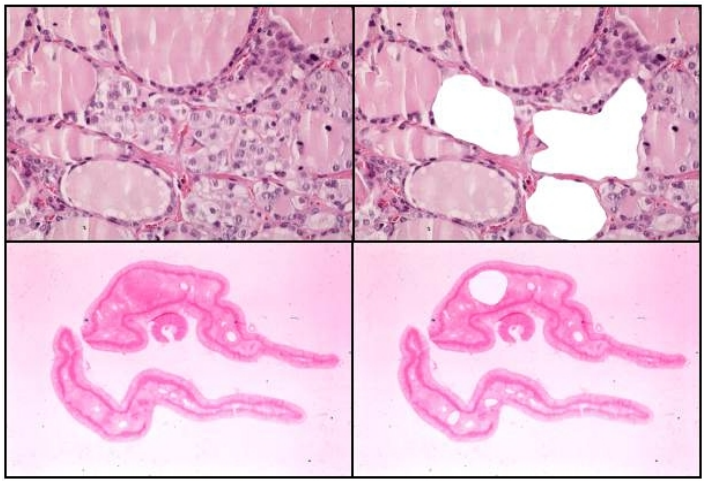

## Design and Methods

- 21 females carrying germline *RET* point mutation (codon 634)
  - 22 CCH foci (1 per lobe and patient)
  - 34 AMH nodules < 1 cm in diameter
- Microsatellite analysis (*TP53, RB1, WT1, NF1*) by optimized PCR-DGGE
- Cell kinetics profile (Ki-67, ISEL, and Ki-67/ ISEL indices) analyzed by microsatellite pattern (stable -MSS- vs. instable -MSI-)

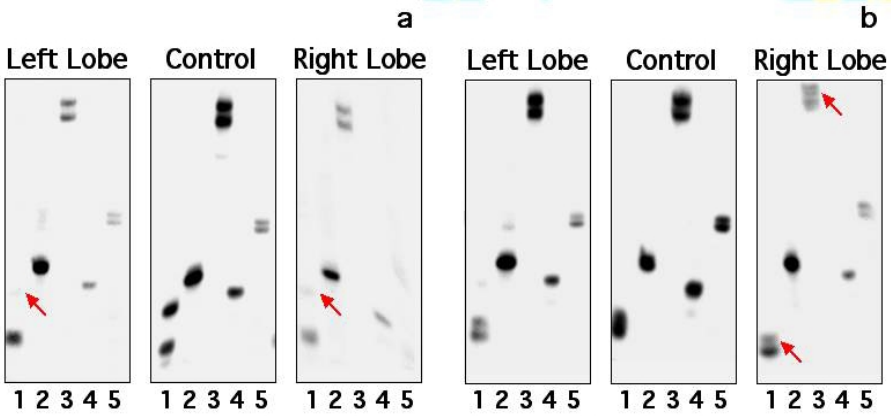
## Patient Distribution




## Microdissection

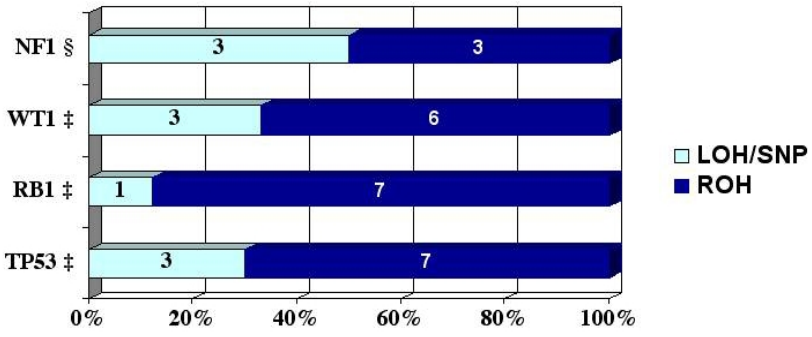


# Microsatellite Patterns in CCH



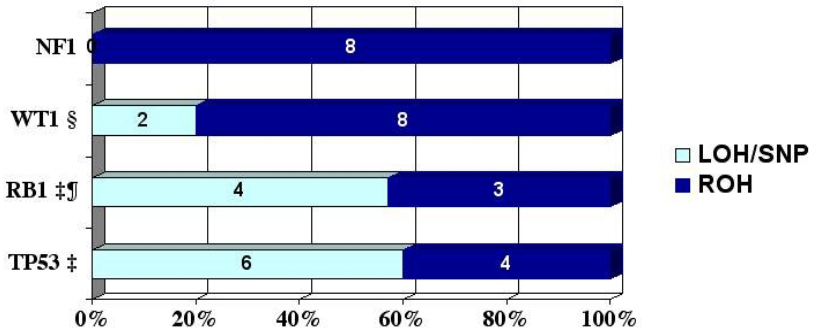

1 & 2 = *TP53*, 3 = *RB1*, 4 = *WT1*, 5 = *NF1*

# Microsatellite Profile in AMH



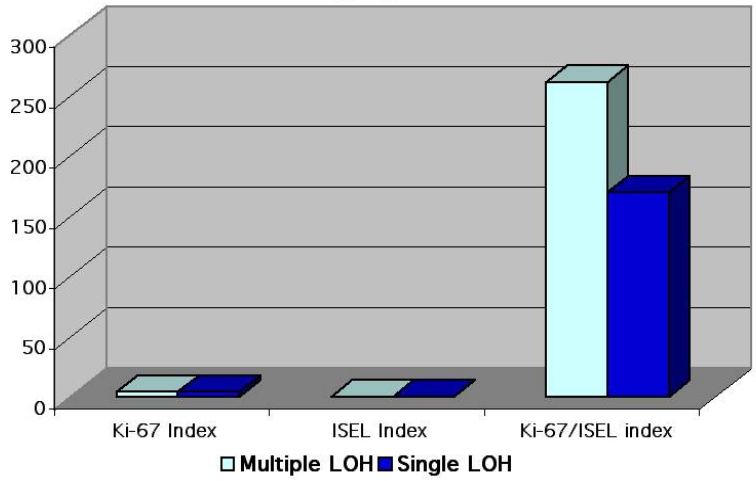

§ = Discordant LOH patterns in nodules from 2 patients (67%).
‡ = Concordant LOH patterns in nodules from 3 patients (42%), but in different TSG in each patient

# Microsatellite Profile in CCH

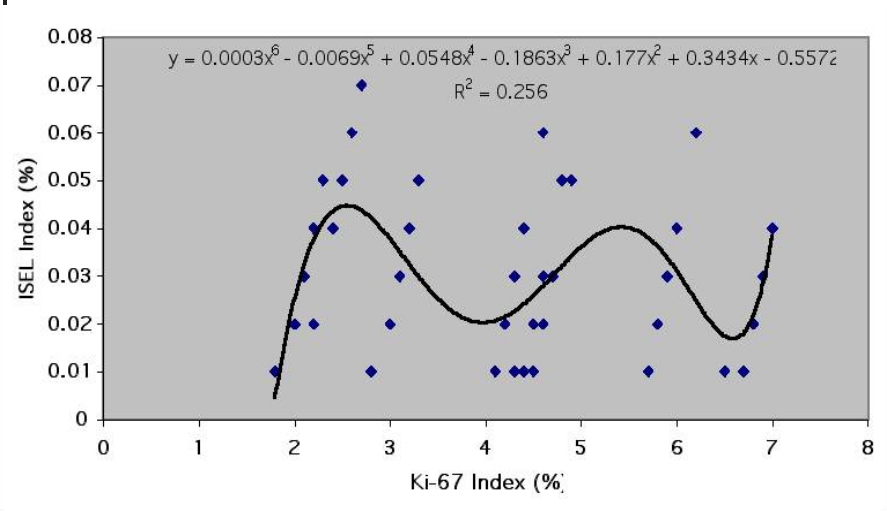



§ = Discordant LOH patterns in both lobes.
‡ = Concordant LOH patterns in both lobes.
¶ = Discordant SNP patterns in both lobes with concordant TP53 LOH patterns in 2 cases.

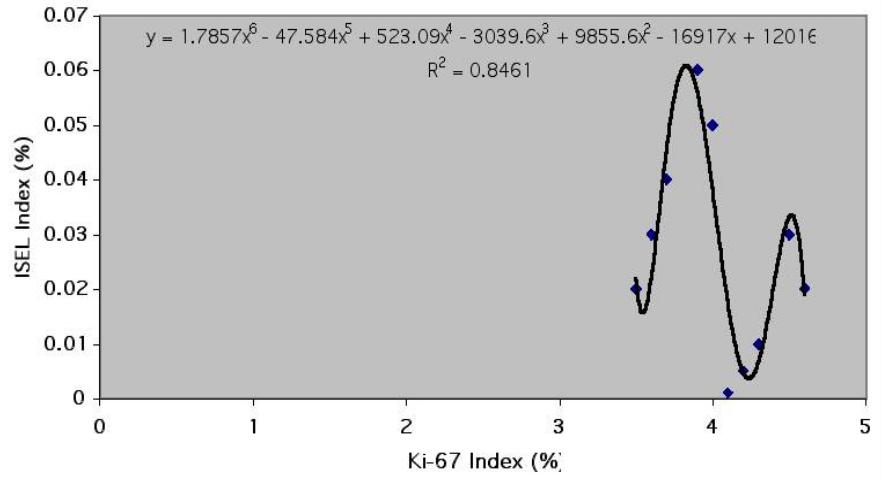
# Average Indices by LOH Profile



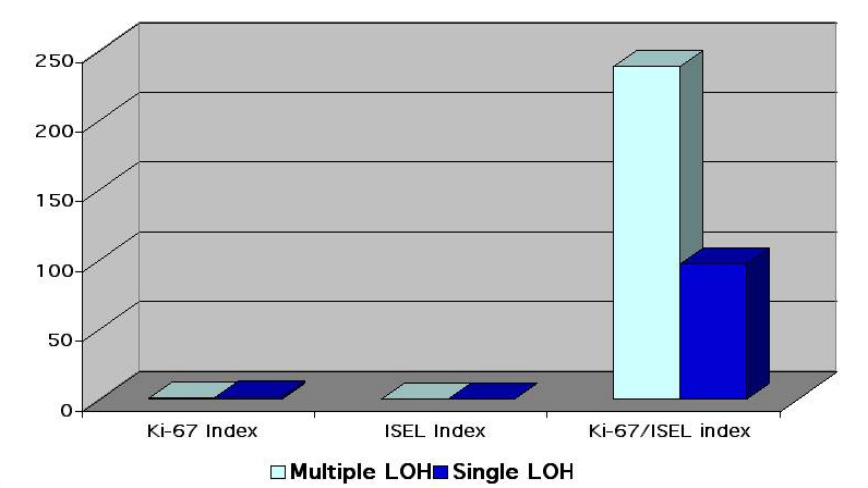
# Proliferation/Apoptosis in MSS Foci



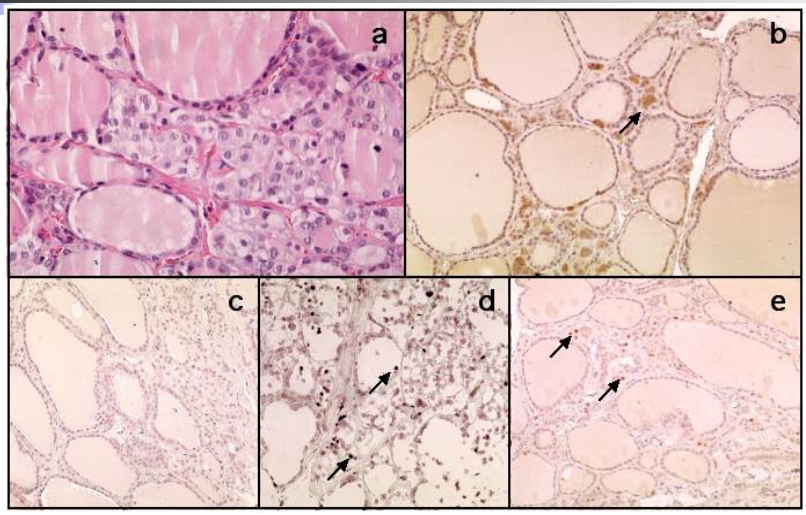

# Proliferation/Apoptosis in MSI Foci



# Kinetic Indices by LOH Profile: Standard Deviation




# CCH in MEN 2A



# Conclusions

- **Microsatellite instable (MSI) MEN 2A lesions reveal the most advantageous kinetic profile, essentially by apoptosis down-regulation**
- **A differential genetic profile contributes to this pattern:**
  - ***TP53/RB1* in CCH**
  - ***NF1* in AMH**