9

INCREASING INTERFERONS (IFNs) EFFECTIVENESS THROUGH AUGMENTATION OF GENE EXPRESSION

Ernest C. Borden, M.D., Dan L. Lindner, M.D., Ph.D., Edward Schwartz, Ph.D.
Greenebaum Cancer Center at the University of Maryland, Baltimore
Albert Einstein University Department of Pharmacology, New York

Only 10 years from their licensure by the FDA for a relatively rare disease entity, hairy cell leukemia, IFNs are now approved worldwide as therapeutic agents for cancer, viral diseases, multiple sclerosis, and specific immunodeficiency syndromes. IFNs result in partial or complete responses in more than a dozen cancers. The clinical challenge over the past several years has been 1) to identify drugs which may augment IFN effectiveness 2) to use IFNs in combination to augment effectiveness of other modalities of treatments. An example of the former (1) is the use of antiestrogens and retinoids to increase effectiveness of IFNs in preclinical models. IFN $\alpha 2$ and IFN β both have augmented antitumor activity in xenografts and antiproliferative activity in for human cell lines when used with retinoids, tamoxifen, or toremifene. For example, in a melanoma cell line, Minor, in which the antiestrogen toremifene had no activity, toremifene increased IFN $\alpha 2$ activity significantly when analyzed by median effect analysis ($p<0.001$). We have shown that this augmentation results from enhanced interferon-stimulated gene (ISG) expression as a result of induction of stat 1 by antiestrogens and retinoids. An example of the latter (2) is the potentiation of 5- fluorouracil (5FU) activity by IFNβ. The combination in randomized Phase II trials of metastatic colorectal carcinoma has been shown to increase survival. In one trial, 37 patients were compared in an unbalanced randomization (2:1). The combination resulted in a survival of 17.6 mos compated to the conrol of 8.2 mos. Both IFN α and IFN β have been shown to increase the expression of the ISG, thymidine phosphorylase, which is a key enzyme in converting 5-FU to its active form, although IFN β has greater effect. Thus molecular pathways leading to augmented ISG expression can increase effectiveness of IFNs in preclinical studies. Randomized clinical trials are underway to test these concepts.

10

A CLINICAL ROLE FOR STEM CELL FACTOR (SCF) IN CANCER PATIENT MANAGEMENT

D Crowther and A Weaver
Cancer Research Campaign, University Department of Medical Oncology, Christie Hospital NHS Trust Manchester M20 4BX UK.

The use of primitive haematopoietic prognenitor cells harvested from the peripheral blood has a well established role in reconstituting haemopoiesis following myeloablative chemo-radiotherapy. G-CSF, GM-CSF and IL-3 have all been used to augment the yield of progenitor cells following chemotherapy. However the requirement to process large amounts of blood and the recognition that some patients are poor mobilisers has led the search for more effective procedures. Stem cell factor (SCF), a glycoprotein haemopoietic growth factor is the ligand for the tyrosinase receptor encoded by c-kit. Definciencies in the SCF-ckit receptor system in mice leads to infertility, mast cell deficiency, defective pigmentation anaemia and dys-megakaryocytopoiesis SCF alone is a survival factor for primitive multilineage progenitor cells but stimulates very little cellular proliferation although it can act synergistically with many haematopoietic growth factors including G-CSF, GM-CSF, Epo and IL-6 resulting in a many fold increase in early progenitor cell populations.

Although SCF is a poor mobiliser when used alone a substantial increase in the yield of blood progenitor cells may be obtained by using SCF in combination with G-CSF. Patients treated with cyclophosphamide 3G/m^2 were randomised to receive G-CSF 5μg/kg alone or G-CSF with increasing doses of SCF (5-20μg/kg). A highly significant increase in yield from a single leukapheresis was obtained with increasing dose of SCF for CFU-GM, BFU-E, CFU-MK and LTC-IC. A median of 3,2L of blood requires processing to provide a target number of 2x10^4 LTC-IC/kg when G-CSF alone is used compared with only 0.83L when 20μg/kg SCF is used in addition. To obtain 2x10^6 /kg CD34+ cells requires a median of 5L of blood to be processed following G-CSF alone but only 1.58L when SCF is used in addition. The methodology is now in place for standardising and improving mobilization of primitive haemopoietic cells to allow multiple myeloablative procedures followed by haematopoietic reconstitution using the product of a single leukapheresis.

11

Hematopoietic Growth Factors in the Treatment of Acute Leukemias The Development of Biologically Defined Concepts

W. Hiddemann, G. Jahns-Streubel, D. Haase, C. Schoch, Ch Fonatsch, B. Wörmann and Th. Büchner.
Dept Hematol & Oncol, Univ of Gottingen, Germany

Hematopoietic growth factors (HGF) are applied for an improved management of acute leukemias. (1) for shortening the duration of critical cytopenia after intensive antileukemic therapy and (2) by priming leukemic blasts for subsequent chemotherapy. While clinical data have demonstrated a beneficial effect of the posttherapeutic application of HGF, clinical results on priming are less convincing. In order to elucidate the mechanisms of HGF stimulation on leukemic blasts and to identify biologic subgroups with different sensitivity to HGF the association of in vitro response to GM-CSF with proliferative activity, parameters of araC metabolism, autonomous cytokine production and karyotype was analyzed in leukemic blasts from 92 patients with newly diagnosed AML.

The patients were divided into 4 groups according to the results of their chromosome analyses: I) normal karyotype (n=39); II) favorable karyotypes {t(8;21),t(15;17), (16)}(n=16). III) unfavorable karyotypes {*inv* (3), -5, del5q, t(6;9)+8, t(9,11), complex abnormalities (3 and more)} (n=20); IV) karyotypes of uncertain prognostic significance (n=17). Proliferative activity of AML blasts differed substantially between four karyotype groups with the highest value being measured in cases with favourable karyotypes. Conversely, sensitivity to GM-CSF was significantly lower for patients with a favourable karyotype (group II) as compared to group I ($p=0.04$) and group III ($p=0.013$) No significant differences between the prognostic groups were found for the parameters of intracellular araC metabolism. The results of the proliferation assays were related to the production of L-1ß, TNF-α, IL-3, IL-6, G-CSF and GM-CSF by leukemic blasts after 24 h of culture in serum-free medium. Samples without spontaneous cytokine production (n=21, group A) had a substantially lower median ^{3}H-TdR incorporation (range;0.0-3,44) as compared to samples with high multi-cytokine production (n=26, group C) ($p=0.062$). For sensitivity to GM-CSF, however median value for group A was 2.6 fold of control (range 1.0-8.0) and thus significantly higher than for group C-2.1 fold of control (0.74-81)($p=0.007$). These results indicate that subgroups of AML with different sensitivity to GM-CSF can be identified and that patients with an unfavorable karyotype and/or a low proliferative activity may benefit from HGF priming. These data furthermore may provide the basis for a more rational application of HGF to biologically defined subgroups of AML patients.

12

TRANSFORMING GROWTH FACTORS ALPHA & BETA - POSITIVE AND NEGATIVE REGULATORS OF EPITHELIAL GROWTH

Robin Leake. Reader in Endocrine Oncology, Biochemistry & Molecular Biology, Davidson building, University of Glasgow, Glasgow G12 8QQ, Scotland.

Transforming growth factor alpha (TGF-α) has long been recognised as the major ligand for the epidermal growth factor receptor (EGFR) in many cancers. The effects of TGF-α are, in most cases, to stimulate both growth and invasion. Blocking the action of this growth factor by either blocking the ligand binding site on the receptor or by inhibiting the receptor-related tyrosine kinase results in dramatic inhibition of growth of breast, ovarian and prostate cancer cells *in vitro*. In ovarian cancer cells that have become resistant to platinum therapy, levels of both TGF-α and the EGFR-related tyrosine kinase are elevated. This makes the use of specific EGFR tyrosine kinase inhibitors a very attractive approach. In contrast, TGF-β down regulates the growth of these cancer cells and loss of sensitivity to TGF-β is thought to be an important step in the progression of individual cancers. However, the role of TGFβ is not so clear cut as that of TGF-α in that TGF-β stimulates the synthesis and secretion of members of the urokinase family. These molecules are normally associated with increased tumour invasion. This presentation will summarise our current understanding of the balance between the two growth factors in terms of regulating solid tumour progression and will attempt to predict the way forward in developing drugs to modulate their actions.