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Glivec for GISTS

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Gastrointestinal Stromal Tumours (GISTS) are rare tumours of the gastrointestinal tract, with a differing level of agressiveness, but always malignant which recur either locoregionally or develop livermetastases. No effective systemic treatment has hitherto been available. GISTS are characterised by an immunohistochemical assay positive for cell surface expression of KIT (CD117). STI 571 (Glivec®) in a small molecule, orally bioavailable drug, that inhibits BCR-ABL leading to responses in CML patients and has also been shown to inhibit KIT.

Two studies have hitherto been reported, a dose finding European study in 36 GIST patients (1) and a randomized phase II study in 84 patients (2).

In the phase I study the maximal tolerated dose appeared 800 mg. Only 4 of the 36 patients progressed in the first 28 weeks. Clinical improvement was observed in 24 of 27 pts (89%). Toxicity mostly mild observed included peripheral edema (52%), fatigue (50%), skin rash (41%), periorbital edema (41%), nausea and vomiting (30%), diarrhea (17%) and anorexia (17%), occurring mostly in the first 8 weeks and later fading away.

In the phase II study at 400 mg a PR of 50% (22/44), SD 28% (12/44) and PD 22% (10/44) and at 800 mg: PR 70% (28/40), SD 25% (10/40) and PD 5% (2/40) were observed.

PET scanning on day 8 in the European study and selected data of the American study herald a clinical response later as will be demonstrated at this meeting by Stroobants et al. An update of the clinical data will be given.

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Induction of differentiation in liposarcomas via PPARgamma-developing a rational therapeutic strategy

Abstract not received.

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The new Melanoma staging system

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A completely revised staging system for melanoma has been approved by the American Joint Committee on Cancer as well as the UICC TNM Committee, the WHO Melanoma Program and the EORTC Melanoma Committee. Major revisions of the new melanoma staging system include: 1) melanoma thickness and ulceration but not level of invasion to be used in the T category (except for T1 melanomas); 2) the number of metastatic lymph nodes and the delineation of clinically occult (i.e.: "microscopic") vs. clinically apparent (i.e.: "macroscopic") nodal metastases to be used in the N category; 3) the site of distant metastases and the presence of elevated serum lactic dehydrogenase (LDH) to be used in the M category; 4) an upstaging of all patients with Stage I, II, and III disease when a primary melanoma is ulcerated. The ability to stage patients more accurately with sentinel node technology was demonstrated by the 20 to 29% differences in 10-year survival for patients with the same TNM criteria who had clinical vs. pathological staging of their lymph nodes. To validate the staging system, thirteen cancer centers and cancer cooperative groups contributed staging and prospective survival data for 17,600 melanoma patients with complete clinical, pathologic and follow-up information. In a multivariate analysis of 13,581 patients with localized melanoma, the two most powerful independent characteristics of the primary melanoma were tumor thickness and ulceration. For 1201 patients with lymph node metastases, the three most significant factors were: 1) the number of metastatic nodes 2) the tumor burden at the time of staging (i.e.: microscopic vs. macroscopic)); and 3) the presence or absence of ulceration of the primary melanoma. Among 1158 Stage IV patients, survival differences were significantly greater for skin, subcutaneous or distant lymph node metastases compared to lung metastases ($p=0.003$) or other visceral sites of metastases ($p<0.0001$). The new staging system, along with a validating multivariate analysis of 17,600 melanoma patients should now form the basis for statistical design and analysis of future melanoma clinical trials that will help determine future standards of care.

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Is there evidence based consensus on adjuvant therapy in stage II–III melanoma?

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Interferons are cytokines with various modulating effects on the inflammatory response. These pleiotropic effects may depend on the concentrations of IFN and include direct and indirect antitumor activities as well as recently recognized anti-angiogenic activity at relatively low doses of IFNalpha. For such a wide range of biologic activities it has been widely recognized that the dose for optimal biologic activity may differ greatly from the maximally tolerated dose. This leads to a difficult position to develop a strategy in clinical development in the absence of clear surrogate endpoints to identify active doses of IFN. It has resulted in a great number of empirical trials, each with its own hypothesis on dose and dose schedule, evaluating a wide range of doses of IFN in a wide range of clinical stages: from melanoma stage II, stage III and mixed stage II–III trials. With all this heterogeneity it comes as no surprise that the outcome of these trials has varied in all stages and has varied almost as widely as the dose range employed. It is remarkable that under these conditions IFN has, for lack of prove of clinical activity, has not been approved for treatment of patients with stage IV melanoma. In spite of this high dose interferon therapy has been approved in the adjuvant setting for high-risk melanoma both in the USA and in Europe, whilst low dose interferon therapy has been approved in Europe but rejected in the USA in the adjuvant setting for intermediate risk melanoma. All this reflects inconsistency in the outcome of trials, reflecting some, but a modest effect of IFN in the adjuvant setting.

Outcomes have been debated vividly but as of yet have not allowed for definitive conclusions on the efficacy or inefficacy of IFN in the management of malignant melanoma as no convincing data have been accumulted on its impact on overall survival in spite of a relatively consistent impact of relapse free survival.

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CD95(APO-1/Fas)-mediated apoptosis: regulation of life and death

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CD95, a member of the tumor necrosis factor (TNF) receptor superfamily induces apoptosis upon receptor oligomerization. The receptor and its ligand are important for apoptosis of peripheral T cells, for downregulation of an immune response and most likely, at least in part, also for peripheral T cell tolerance. In Aids, apoptosis mediated by this system might contribute to the depletion of T helper lymphocytes. Likewise, in diseases in which liver cells are destroyed the CD95 system might play a major role.

In a search to identify the intracellular signalling pathway of CD95 several molecules coupling to oligomerized CD95 were immunoprecipitated from apoptosis-sensitive human leukemic T cell and lymphoblastoid B cell lines. The following binding molecules were only associated with aggregated and not with monomeric CD95: phosphorylated FADD (MORT1) and caspase 8. Thus, caspase 8 was identified as the most CD95 receptor proximal protease which starts the cascade of protease reactions important for CD95-mediated apoptosis. Association of FADD and caspase 8 with CD95 was not observed with C-terminally truncated non-signalling CD95. FADD and FLICE did also not associate with a CD95 cytoplasmic tail carrying the lprcg amino acid replacement. FADD and caspase 8 form a death-inducing signalling complex (DISC) with the CD95 receptor and are, thus, the first CD95 associating proteins of a signalling cascade mediating apoptosis.The function of the DISC is discussed in detail, particularly with respect to its role in sensitivity and resistance to apoptosis.

The CD95 death system plays a role in destruction of liver tissue. In hepatitis cytotoxic T lymphocytes might use the CD95 system to kill infected hepatocytes. In M. Wilson copper overload leads to upregulation of the CD95 ligand that may finally contribute to acute liver failure. In HCC from patients treated with chemotherapeutic drugs the CD95 receptor and ligand are upregulated and may contribute to apoptosis of the tumor or, dependent on the drug sensitivity of the tumor, to the status of the tumor as an immunoprivileged site.

References

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[3] P.H. Krammer, Tumorimmunology Program, German Cancer Research Center, Heidelberg, Germany

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Radiation response and apoptosis

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Apoptosis or programmed cell death is a distinct mode of cell destruction and represents a major regulatory mechanism for removing abundant and unwanted cells during embryonic development, growth, differentiation and normal cell turnover. Failure to eliminate cells that have been exposed to mutagenic agents has been associated with the development of cancer and resistance to anticancer therapy.

Ionizing radiation, like most chemotherapeutic agents, induces apoptosis in a wide variety of cell systems. The magnitude of this response, however, depends to a large extent on the cell type and the dynamic balance between survival and apoptosis-promoting signals. An essential step in the execution phase of the apoptotic death program involves the sequential cleavage and activation of a group of cysteine proteases (caspases). Radiation may activate this caspase cascade in different subcellular compartments and by different mechanisms. Upon its activation by DNA-damaging agents, the tumor suppressor p53 can act as a direct transcriptional regulator of bax, a pro-apoptotic member of the Bcl-2 family. P53-dependent upregulation of death receptor/ligand systems provides another link between radiation-induced DNA damage and the apoptotic machinery. At the mitochondrial level radiation-induced reactive oxygen intermediates have been shown to mediate the release of cytochrome c and subsequent caspase activation. Finally, plasma membrane-derived lipid second messengers, activating stress-induced signal transduction pathways may also target the caspase cascade.

The potential radiobiological and clinical relevance of radiation-induced apoptosis is illustrated by several lines of experimental evidence: (1) overexpression of apoptosis-promoting or -suppressing genes modifies radiation-induced clonogenic cell survival and radiosensitivity, (2) the cellular propensity to undergo apoptosis is maintained throughout fractionated radiation schedules, (3) in certain tumors pretreatment levels of apoptosis have been shown to predict for clinical outcome after radiotherapy.

In summary, radiation-induced apoptosis has been a focus of intense research during the last decade. Its recognition as a significant component of radiation-induced cell death and potential co-determinant of radiosensitivity has initiated several lines of research aimed at modulating the apoptotic response in both tumor and normal cells in order to increase the radiotherapeutic ratio.

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More than one way to die: apoptosis and necrosis induced by death domain receptors

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Apoptosis and necrosis are two distinct forms of cell death. In an in vitro cell culture model system of death domain receptor induced cell death, we have compared morphological and signal transduction events occurring during Fas-ligand induced apoptosis and TNF-induced necrosis in the same cellular context of the L929sA fibrosarcoma cell line. Caspases are indispensable as initiators and effectors of apoptotic cell death and are involved in many of the morphological and biochemical features of apoptosis. Major changes in mitochondrial membrane integrity and release of proapoptotic factors, such as cytochrome c from the mitochondrial intermembrane space, play an important sensor and amplifying role during apoptotic cell death. Necrosis is not correlated with active caspases, cytochrome c release or internucleosomal DNA fragmentation. Principal elements of necrosis include mitochondrial oxidative phosphorylation, reactive oxygen production, and non-caspase proteolytic cascades depending on serine proteases, calpains, or cathepsins. Inhibition of the classical caspase-dependent apoptotic in several cell lines pathway leads to necrotic cell death. Thus, the same cell death stimulus can result either in apoptotic or necrotic cell death, depending on the availability of activated caspase. Therefore, death domain receptors may initiate an active caspase-independent necrotic signaling pathway. Also the differential interrelation between apoptotic and necrotic cells and macrophages will be discussed.

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Apoptosis in the normal and malignant colon

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The question is intruiging as to why the normal epithelia of the gut are very sensitive to chemotherapeutic drugs, whilst carcinomas of the colon are resolutely resistant to drugs like 5-fluorouracil (5-FU) or Tomudex. Our experiments suggest that the "survival threshold" of normal epithelial cells is set by a number of key genes which control apoptosis and progress through the cell cycle. This threshold determines how easy or difficult it is for a cell to die after drug-induced damage. For example, 5-FU toxicity to mouse small intestinal epithelia is lost in an animal in which the tumour suppressor gene p53 has been deleted. Both functions of p53, as a pro-apoptotic protein and as an initiator of cell cycle delay, via p53-stimulated expression of the cyclin-dependent kinase inhibitor p21,are required for the expression of toxicity. Loss of p53 in colon tumors indicates a poor prognosis. Mice in which p21 has been deleted show an attenuated response to 5-FU.

Interestingly, the pure thymidylate synthase inhibitor Tomudex, considered to have mechanistic similarities to 5-FU, and which is used to treat colon carcinoma, has a p53-independent mode of action. Gut epithelia from mice of different strains differed profoundly in their response to Tomudex, suggesting that it is possible to use genetic methods to estimate the numbers of genes which set the "survival threshold" and to isolate those responsible for p53-independent cells death. These genes are likely to be altered in late stage p53 mutated/deleted tumours and to provide the gateway to effective therapies of an otherwise inherently chemo-resistant tumour.

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Regulation of p73 in cell death

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The p53-homologue p73, has been mapped to a region (1p36.33) which is frequently deleted in neuroblastomas, suggesting that its alterations may play a role in the development of tumours of the nervous system. However, unlike p53, mutant p73 has rarely been found in human tumours. Few studies have directly investigated functions of p73, and their activities have been largely assumed based on their structural similarities with the p53 homologue. p73 has indeed been shown to induce apoptosis when transfected into cells. Here we show that:

(1) p73 is expressed as distinct forms at the C-terminus (spliced forms, α, β, γ, δ, ε and ζ) and N-terminus (two distinct promoters for TA-p73 and Delta-N-p73), which show different abilities to homo/hetero-dimerize and transactivate target promoters.
(2) DNA damaging agents, through MLH1 and c-Abl, increase the half-life of p73. This pathway is independent and parallel to p53, being relevant for cancer development, progression, and therapy.
(3) p53 regulates transcriptionally Delta-N-p73, which in turn functionally inactivates p53 (loop).
(4) p73δ is induced during keratinocyte differentiation, while p63/ΔNp63 are repressed. Both p73δ/p63 transactivate differentiation specific promoters.
(5) p73 trigger neuronal differentiation: p73 expression is upregulated during neuroblastoma differentiation. Overexpression of p73 (not A156V mutants or p73Δ84) trigger neuronal differentiation (regulating MYCN, NCAM, pRB). Dominat negative 73Δ84 blocks NCAM transactivation induced by retinoids.

In conclusion, p73 seems involved both in tumour suppression and development.