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Interferon-induced STAT 1 activation in malignant melanoma cells and tumor tissues

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Background: STAT 1, a member of signal transducers and transcription activators of STAT family proteins, has been implicated as important mediator of interferon (IFN) signaling. Functional activation of STAT 1 requires tyrosine and serine phosphorylation. Defects in its expression or activation in response to IFNs were observed in numerous pathological conditions including cancer.

Purpose: To explore cancer-associated impaired STAT 1 response to IFNs that may in clinical situation affect sensitivity of malignant melanoma to immunotherapy.

Methods: The inducibility of serine (S 727) and tyrosine (Y 701) phosphorylation by IFN-α/γ was assessed in 21 melanoma cell lines and in 35 primary cultures derived from melanoma patients. STAT 1 levels and inducibility of its activated phosphoforms were detected by Western blot analysis using specific polyclonal and monoclonal antibodies.

Results: All cell lines as well as patient melanoma samples expressed STAT 1 with variable signal intensity. Significant impaired IFN-induced STAT 1 S 727 phosphorylation was observed in both model systems with average of 77% of non-responders recorded in patient melanoma cells and 76% in melanoma cell lines. Failure of PY 701 induction occurred in patient samples (63% after IFN-α and 34% after IFN-γ induction) and in a lesser degree in cell lines (i.e. response absence to IFN-α in 5 and to IFN-γ in 2 melanoma lines). On the basis of detail analyses of patient melanoma cells with respect to the inducibility of STAT 1 phosphorylation by IFNs, four categories of patients could be distinguished: a) activation on both S 727 and Y 701, b) not inducible response, c) activation on Y 701 but not on S 727, d) heterogeneous response.

Conclusions: The study clearly demonstrates STAT 1 functional abnormalities in melanoma cells. Clinical study is now in progress to establish the significance of *in vitro* STAT 1 activation for predicting the patients response to IFN-based therapy and to explore biological consequences in cases responding *in vitro* to IFN-induced STAT 1 activation on only one of the critical amino acid residues.

The study was supported in part by Grant NC/7139-3 from the Internal Grant Agency of the Czech Ministry of Health and by Grant 301/03/0370 from the Grant Agency of the Czech Republic.

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Multicenter phase III randomized trial of polychemotherapy (CVD regimen) versus the same chemotherapy (CT) plus subcutaneous (SC) interleukin-2 (IL-2) and interferon-alpha-2b (IFN) in metastatic melanoma.

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Background: Systemic medical management has failed to significantly improvethe survival of patients with metastatic melanoma. The results of the currently available therapies are controversial and the combined biochemotherapy seems to be interesting. We performed a phase III study using out-patient concurrent biochemotherapy (BC) versus CT as first-line approach in order to compare the two treatments in terms of overall survival (OS), other than response rate (RR), time to progression (TTP), and toxicity.

Material and Methods: From 02/99 to 12/02, 147 pts were enrolled (8 of whom ineligible) with the following characteristics: stage IV non-choroidal melanoma; ECOG performance status (PS) 0-2; absence of brain metastases. Pts were stratified according to site of metastases (visceral vs soft tissues) and prior adjuvant IFN therapy. CVD consisted of Cisplatin 30 mg/m^2 (days 1-3), Vindesine 2.5 mg/m^2 (only day 1), DTIC 250 mg/m^2 (days 1-3), where BC consisted of the same CT plus IL-2 9 MIU sc (days 1-5, 8-12) and IFN±2b 5 MU/m^2 sc (days 1-5). The whole treatment was repeated every 21 days and response was assessed every 2 cycles. 69 and 70 pts receiving CVD (arm A) and BC (arm B), respectively. The Pts characteristics were: median age 50 yrs old (range 19-70), sex (89 males and 50 females) and PS (0 in 136 pts, 2 in one pt and missing for 2 pts). Then, the visceral/nonvisceral site ratio was 100/39. Forty pts were previously treated with adjuvant IFNα.

Results: By intent to treat analysis the RR was 21% (95% CI:13-31%) for arm A vs 27% (95% CI: 18-38%) for arm B, with 3% of complete responses only in BC arm. The median TTP for arm A was 6 vs 7 mos for arm B (p=0.7706) and the median OS was 12 mos for arm A vs 11 mos for arm B (p=0.7519). The main side effects observed in BC arm were 36% of G3 fever and 20% of G3-4 asthenia.

Conclusions: Complexively, the toxicity was manageable in out patient setting and the feared vascular leak syndrome associated with iv high dose IL-2, with the consequent pulmonary edema and kidney failure, was not observed.

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Randomised trial of dacarbazine versus BOLD chemotherapy combined with natural or recombinant alfa-interferon in patients with advanced melanoma

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Background: Polychemotherapy regimens have yielded higher response rates than the standard agent dacarbazine (DTIC) in advanced melanoma in some studies. α-interferon (IFN) may act synergistically with chemotherapy. This randomised phase II study compared the efficacy of DTIC and BOLD chemotherapy combined with either natural α IFN (Finnferon-alpha® or recombinant α IFN-2b (Introna® in patients with advanced melanoma.

Patients and methods: 108 patients were enrolled, of whom 106 were eligible and formed the basis for the efficacy analyses. 56% of the patients had abdominal visceral and/or bone involvement. Patients were randomised to receive A) DTIC plus Finnferon-alpha® (n=25), B) BOLD plus Finnferon-alpha® (n=31), C) DTIC plus Introna® (n=25) or D) BOLD plus Introna® (n=25). The dose of DTIC was 250 mg/m^2 i.v. days 1-5. The BOLD regimen contained DTIC 200 mg/m^2 i.v. days 1-5, vincristine 1 mg/m^2 i.v. days 1 and 4, bleomycin 15 mg i.v. days 2 and 5 and CCNU 80 mg orally day 1. The dose of IFN was 3x10^6 IU s.c. daily starting on day 8 for 6 weeks and 6x10^6 IU three times weekly thereafter.

Results: Overall response rates were: arm A: 8%, arm B: 13%, arm C: 12% and arm D: 24%. The differences were not statistically significant. However, there was a trend in favour of BOLD plus recombinant α IFN-2b. The BOLD arms produced 6 out of 8 complete responses (CR). All CRs occurred in the patients with metastases limited to the soft tissue and/or lung. The median duration of CR was 46.1 months. The median survival was 9.0 months (range 0.5-84.6+) and the median time to progression was 3.2 months (range 0.2-73.3+). The differences in survival and time to progression between the arms were insignificant. The median survival was 11.1 months with arm A, 9.8 months with arm B, 9.1 months with arm C and 7.5 months with arm D (P=0.62). BOLD was more toxic than DTIC.

Conclusions: There were no statistically significant differences in efficacy between the four treatment arms in patients with advanced melanoma but the small sample size precludes definite conclusions. However, there was a trend towards higher response rate with BOLD plus recombinant αIFN-2b. The patients with soft tissue and/or lung metastases may achieve more CRs with the BOLD regimens than with the DTIC schedules whereas the patients with abdominal visceral and/or bone involvement derive little benefit from the tested regimens.

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Hypoxia-inducible factors 1a and 2a in skin malignant melanomas

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Background: Hypoxia Inducible Factors 1a and 2a are a key transcription factors activating angiogenesis, glycolysis and cell migration as a response to hypoxia.

Methods: HIF 1a and HIF 2a cytoplasmic and nuclear expression was assessed immunohistochemically (ESEE122 and EP190b MoAbs) in a series of 46 nodular malignant melanomas of the skin treated with wide local excision. The expression of VEGF was also examined with VG1 MoAb.

Further associations were sought with patient prognosis, and the important histopathological features of Breslow's thickness, Clarke's levels of invasion, mitotic rate, inflammatory cell infiltrates and tumor ulceration.

Results: HIF1a and HIF2a expression by melanoma cells was correlated directly with VEGF expression. HIF1a expression was more frequent in cases with low mitotic index, but there was no association of HIFas with other histopathological variables. Tumors having high VEGF or HIF2a expression were associated with a poorer prognosis in both univariate and multivariate analysis. The value of Breslow's thickness and Clarke's levels in prognosis was reaffirmed, although only in univariate analysis.

Conclusions: Overexpression of HIF1a and HIF2a are linked to VEGF expression in nodular malignant melanomas. HIF2a and VEGF are important prognostic factors in melanoma.

Genitourinary cancer

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Quality assurance in conformal prostate radiation therapy: technology questionnaire for EORTC trial 22991

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For the EORTC Radiotherapy Group Purpose: A technology questionnaire is designed to evaluate the abilities of participating centres to comply with the required procedures in EORTC trial 22991 for high dose three-dimensional conformal radiotherapy (3D-CRT).

Materials/Methods: Questionnaires on imaging (CT) data acquisition, treatment planning, delivery and verification systems and data transfer systems were sent to the participating centres (n=31). Over 50 questions covered the technical infrastructure used for the compulsory and optional procedures described in the protocol. The frequency of basic verification and calibration of geometrical and mechanical parameters was documented as well.

Results: All centres replied to the questionnaire. The vast majority (>95%) of questions was completed. All centres have appropriate CT slice thickness and matrix size to acquire images with good resolution and to allow satisfactory 3D reconstruction. All centres use beams eye views to shape treatment fields and all but 2 produce digitally reconstructed radiographs (DRR) to display treatment fields. All centres can generate dose-volume histograms of the organs of interest. 3D dose calculation algorithms are available in all centres except one. The data including DRR, isodose charts and treatment reports can be transported by hard copy or image format in more than half of the centres and rarely by DICOM or by manufacturer specific format. Treatment verification is done by only conventional portal imaging (6), EPID (11) and both (14). In vivo dosimetry, optional in this trial, is done with only diodes (6), only TLD (1) or more than one option (7). There is a variation in the frequency of the QA procedure of external beam units, however the results are in accordance with the international accepted tolerance levels and practice guidelines.

Conclusion: The results of questionnaire confirm that participating centres have access to the relevant equipment and can cope with the procedures which are necessary to deliver 3D-CRT properly according to the trial recommendations.

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Prognostic factors in advanced nonseminomatous germ cell tumors (NSGCT): importance of primary tumor (PT) histology and numbers of negative prognostic factors (NPF).

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Purpose: Generally accepted IGCCCG classification has some limitations the main of which is the absence of data about histology subtypes. In our study we retrospectively studied prognostic relevance histology as well as other factors in advanced NSGCT.

Patients and methods: 482 NSGCT CT-naïve pts treated with modern etoposide-based CT in our department during 1987 2001 were included in the analysis. Median f.-up time was 39 months. The end-point of the study was overall survival (OS). Cox regression analysis was used on these data. All markers levels were defined as *log* of absolute values.

Results: Multivariate analysis revealed the following prognostic factors as independent: presents of teratoma in PT (hazard ratio (HR) 1.59; 95% confidence interval (CI), 1.04-2.44), ±-fetoprotein (AFP) level (HR 1.14; 95% CI, 1.06-1.23), lactate dehydrogenase (LDH) level (HR 1.56; 95% CI, 1.20-2.01), presence of nonpulmonary visceral metastases (NPVM) (HR 2.23; 95% CI, 1.32-3.77), and mediastinal PT (HR 3.17; 95% CI, 1.44-7.00). 3 prognostic groups were distinguished: good prognosis (testis/retroperitoneal PT, absence of teratoma in PT, AFP level < 1000ng/ml and LDH level < 1,5 x upper limit of normal (ULN) range); intermediate prognosis (testis/retroperitoneal PT, presence of teratoma in PT, either AFP level ≤10000ng/ml, LDH level 1,5-10xULN or AFP level 1000-10000ng/ml, LDH level ≤ 10xULN); poor prognosis (either mediastinal PT, presence of NPVM, AFP level >10000ng/ml or LDH level >10xULN). Depending on the number of NPF intermediate and poor prognostic groups can be divided into two (see table).

Proposed prognostic groups	No. of pts	3-years OS (95% CI)	1 NPF 3-years OS (95% CI)	2 NPF 3-years OS (95% CI)	3 NPF 3-years OS (95% CI)
Good	21%	92% (87 - 98)	–	–	–
Intermediate	59%	76% (73-82)	83% (75-89)	69% (45-83)	50% (29-71)
Poor	20%	45% (34-55)	49% (37-61)	28% (7-49)	–

Conclusions: The analysis showed negative prognostic significance of teratoma elements in PT and numbers of NPF for advanced NSGCT pts. It should be taken into account for planning of treatment of these pts.

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The effect of total radiation dose and overall treatment time on local control for bladder cancer

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Purpose: To evaluate the influence of total radiation dose and overall tratment time on local control in patients with T2, T3 bladder cancer treated with radical radiotherapy.

Material and Methods: The analysis is based on 480 patients with T2, T3 bladder cancer, who were irradiated at the Center of Oncology in Gliwice between 1975 and 1995. There were 35% of patients with T2 and 65% of patients with clinical stage T3 bladder cancer. Radiotherapy was performed with photons X 9-23 MV or Co^{60}. Total radiation dose (TD) ranged from 59,2 Gy to 72 Gy (mean- 65.5 Gy), overall treatment time (OTT) ranged from 30 to 91 days (mean- 51 days). The fractionation schedules were as follows: **1.** conventional fractionation (once a-day, dose per fraction of 1.8-2.0 Gy, mean TD- 65.4 Gy, mean OTT- 53 days), **2.** protracted fractionation (once a-day, dose per fraction of 1.6-1.7 Gy to pelvis and dose per fraction of 2.0 Gy to boost, mean TD- 65.4 Gy, mean OTT- 62 days), **3.** accelerated boost (pelvis irradiated once a-day with dose per fraction of 2.0 Gy, boost twice a-day with dose per fraction of 1.3-1.4 Gy, mean TD- 66 Gy, mean OTT- 45 days), **4.** accelerated hyperfractionation (both pelvis and boost irradiated twice a-day, dose per fraction of 1.2-1.5 Gy, mean TD- 65.6 Gy, mean OTT- 41 days). In 261 patients (54%) there were planned and unplanned gaps during radiotherapy. Local control probability was estimated using the logistic regression with application of LQ-model. In the second step of the analysis the Cox regression was used.

Results: With the median follow-up of 76 months, the actuarial 5-year local control rate was 47%. In both the logistic regression and the Cox regression, total radiation dose (p=0,01 and p=0,009, respectively) and overall treatment time (p=0,03 and p=0,04, respectively) were independently correlated with local control. Using the logistic regression the estimated time factor was 0.21 Gy/day, which means that prolongation of overall treatment time for one day requires the extra dose of 0.21 Gy for the same probability of local control.

Conclusions: This study demonstrates that total radiation dose and overall treatment time might be the important factors for local control in bladder cancer.