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T92-0045: Interlaboratory quality control on Tpot measurements.

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Purpose/objective:
To assess the reproducibility of Tpot measurement by comparison between three laboratories.
Materials and methods:
We will report on the intercomparison which contains two arms: a single set of data (*disc* analysis), stained, processed and analysed in Lab1 has subsequently been analyzed by the team in the Lab 2 and in Lab 3. This kind of comparison reveals differences in interpretation and region setting. Pieces of the original tumor specimen have been processed, stained and analyzed separately in each centre (*meat* analysis). This latter reveals variation in dissociation, staining and running the sample, but also illustrates tumor heterogeneity. All three laboratories are equipped with a Becton Dickinson FACScan and are using PC-Lysis for analysis. The procedure for handling the sample has been standardized before starting the comparison; guidelines were elaborated for setting the gates. The mathematical algorithm modified from A. Begg has been used. The study consists of 102 specimens from 97 patients with following breakdown: 25 gynecological, 36 head and neck, 35 rectal and 6 pulmonary cancers. In order to compare Tpot-data the method of Bland and Altman has been used which yields limits of agreement. This method gives a better impression on the true correlation between centers as compared to the correlation coefficients. Moreover, it results in a closer estimate of the variation on an individual specimen. The analysis has been done on the 102 specimens but a second analysis has been performed on 89 biopsies, after having excluded outliers, with obvious aberrant values.
Results:
The Bland and Altman analysis of log Tpot for all 102 samples yields small mean differences (range of logdata 0.004 to 0.151), but large standard deviations (range 0.286 to 0.407 in logdata). Converting the logdata to days yields a mean difference of 1 - day and a standard deviation ranging from 1.9 to 2.6 days. Restricting the analysis to 89 samples (excluding obvious aberrant outliers), improves the standard deviation to a range of 0.161 - 0.326. This results in a standard deviation of 1.45 - 2.1 days. This improvement is only observed for *disc* data and not for *meat* data.
Conclusions:
Comparison of on disc yields good agreement after removal of a few obviously aberrant results. The lack of improvement for meat data after having removed aberant results might reflect tumor heterogeneity.

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AN ANTI-ANGIOGENIC AGENT (TNP-470) INHIBITED REOXYGENATION DURING FRACTIONATED RADIOTHERAPY OF MURINE MAMMARY CARCINOMA

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Purpose: Angiogenesis is one of the important factors for tumor growth. Therefore, an angiogenesis inhibitor might decelerate tumor repopulation and is expected to improve the tumor control rate in fractionated radiotherapy (RT). On the other hand, it might increase hypoxic fraction of tumors or inhibit tumor reoxygenation during fractionated RT. This study investigated the effects of an angiogenesis inhibitor on fractionated RT.
Materials and Methods: Animal-tumors were early generation isotransplants of mammary carcinoma in C3H/He mice. Tumor response was studied by tumor growth (TG) time and TCD-50 (50% tumor control dose) assays. Treatments were started when tumors on the right paw grew 4-5 mm in diameter. Radiation was locally given to tumors in air or under hypoxic condition. An angiogenesis inhibitor, TNP-470, a synthetic analogue of fumagillin which is a natural product of *Aspergillus fumigatus*, has been reported to inhibit endothelial cell growth *in vitro*. TNP-470 was administered s.c. twice a week at a dose of 100mg/kg. In the TG time assay, fractionated RT was delivered daily for 5 days to a total dose of 10Gy (2Gy/fraction x 5). Two or four doses of TNP-470 were administered during and/or after fractionated RT. The time required for a tumor to reach 3-fold of initial tumor volume (TG time) was determined for each group. In the TCD-50 assay, a single or fractionated irradiation was given alone or in combination with TNP-470. Fractionated irradiation was delivered daily, five times per week, over two weeks (10 fractions). One dose of TNP-470 was administered 24 h prior to a single dose of irradiation, whereas four doses of TNP-470 were given during fractionated RT. Tumors were observed for recurrence once a week for 120 days following the end of RT.
Results: The TG time for no treatment group, a group treated with fractionated RT alone, or two doses of TNP-470 alone was 5.3 days (95% confidence limits: 4.8-5.9), 15.6 days (15.1-16.1) or 7.8 days (7.1-8.5), respectively. Significant delay of tumor growth was observed by TNP-470 alone (100mg/kg x 2), indicating that TNP-470 alone has antitumor effect *in vivo* ($p < 0.001$). This antitumor effect of TNP-470 was not observed when this drug was given during fractionated RT. However, TNP-470 given after the end of fractionated RT showed significantly additional growth delay. The values of TCD-50 are as follows;

Treatment condition		TCD50 (Gy) (95% confidence limits)		
		RT + TNP-470	RT alone	difference
Single dose	in air	53.1 (49.9 - 56.5)	49.4 (46.5 - 52.5)	NS
	hypoxic	56.8 (56.3 - 57.3)	54.7 (52.4 - 57.1)	NS
10 fractions	in air	77.3 (73.5 - 81.3)	67.4 (65.2 - 69.7)	$p < 0.005$
	hypoxic	124.5 (118.7 - 130.7)	121.1 (116.6 - 125.8)	NS

TNP-470 given prior to RT did not alter hypoxic fraction of tumors significantly. TNP-470 administered during fractionated RT decreased radiocurability of the tumors in air, although no difference in TCD50 was observed under hypoxic condition. It suggested that TNP-470 inhibited reoxygenation during 10-fraction RT.

Conclusions: TNP-470 showed additional tumor growth delay in the TG time assay, only when it was given after the whole course of fractionated RT. In the TCD-50 assay, administration of TNP-470 during 10 fractions of RT decreased the local tumor control rate. These results suggest that reoxygenation during fractionated RT might be inhibited by TNP-470. When TNP-470 is combined with fractionated RT, administration after the end of RT is recommended.