

influence transcriptional and/or translational efficiency or mRNA stability. Response to 5-FU also depends on TS structure and some researchers showed that variant structural forms of TS in tumour cell lines confer resistance to fluoropyrimidines. We intend to find any relationship between the polymorphisms in the 5' and 3' UTR with the TS levels and to analyze the whole coding regions of the TS gene.

**Materials and Methods:** We performed the TS-DNA gene sequence in 68 colorectal cancer (CRC) samples from patients of different Dukes' stages (A, B, C). The 5' UTR polymorphism was evaluated amplifying DNA by PCR, amplification products were electrophoresed in 3% agarose gel. Products of 116 bp (2R/2R), and 144 bp (3R/3R) or both (2R/3R), depending on the TS genotype were obtained. The 3' UTR analysis was carried out by RFLP. The quantification of TS expression, was obtained by Light Cycler- TS mRNA quantification Kit (Roche). The sequence of the exons was performed amplified every exon by PCR and sequencing them by the SequiTherm EXCEL II DNA sequencing kit on a LI-COR sequencer. **Results:** Significantly higher values of TS mRNA were found in the 3R/3R group and 2R/3R group compared with 2R/2R respectively. No significant association was found for the polymorphism of the 3' UTR and the TS mRNA levels. The sequencing of the 7 exons of the gene did not show any mutation.

**Conclusions:** 5-FU improves survival in a subgroup of CRC patients but predictive markers are required to identify patients that benefit from such treatment. Low intratumoral TS levels are associated with a good response to chemotherapy, so patients with the genotype 2R/2R, associated with lower TS levels, could have a better prognosis and a better response to 5-Fu. Because of missing mutation in the TS exons we intend to widen our study to Dukes' metastatic CRCs that in spite of their high genomic instability could present mutations explaining their frequent 5-FU drug resistance, and a worse prognosis.

#### 400 POSTER Measurements of residual radiation spectra from neutron activation in a medical linear accelerator

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**Background:** Linear accelerators are known to produce neutrons by the atomic reaction (g,n). It is of an interest to measure the residual spectra of activated materials in the treatment rooms.

**Method and Materials:** Measurements were done in two treatment rooms in two clinical facilities. The accelerators used were both Elekta Synergy S units with beam modulators. The measurements were done with 6 MV and 18 MV photon energies on one accelerator and with 6 MV, 10 MV, and 15 MV on the other. A collimated scintillation BGO detector (Bismuth Germinate or Bi4Ge3O12) was used. The detector is connected to a Multi-Channel Analyzer (MCA), which is in turn connected to a laptop equipped with an QtmMCA software. The detector was calibrated using Co57, Ba133, Cs137 sources. An Ir192 was placed in the detection area for reference.

**Results:** For both accelerators, there was no observable change in the spectra before and after the 6 MV irradiation. However, following the 10 MV, 15 MV, and 18 MV, residual radiation was detected. For 10 MV, there was an activation peak with half life of about 134 seconds. For 15 MV, the peak half life was about 114 seconds. For 18 MV, there was a significant peak at about 150 KeV with half life of about 145 sec. The suspected isotope is one of the following: Sr79, Fr87, Cd48, Pm61, Pr59, Os66. Another peak was about 1.5 MeV, which probably belongs to Al28.

**Conclusion:** There is no significant activation from 6 MV photon beam. There is a significant activation following 10 MV, 15 MV, and 18 MV beams with relatively long half live, 134 sec, 114 sec, and 145 sec, respectively. It should be taken into consideration when department policies are written. When using mixed beams set up, it should be preferred to treat the high energy beams first.

#### 401 POSTER Methylation and chromosomal losses in squamous cell carcinoma of the head and neck

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**Background:** DNA methylation is essential for normal differentiation and development and aberrant DNA promoter methylation is a common feature of many human cancers. Another thing is unilateral chromosomal losses important role in Head and Neck cancer. We investigate the extent of chromosomal losses and the status of CpG methylation in Head and Neck cancer in relation with the clinicopathologic factors.

**Material and Methods:** Normal mucosa and tumor tissue 17 cases were examined with a methylation-specific PCR on 15 cancer-linked genes

and a total of 29 cases were examined with a PCR-based loss of heterozygosity (LOH) analysis using a panel of 41 microsatellite markers on 8 chromosomes.

**Results:** The pattern of methylation changes between the paired normal mucosa and tumor site was variable the total of 206 cases examined for the methylation status of non-CpG island showed 34 cases hypomethylation changes, 26 cases hypermethylation changes, 31 cases no methylation changes and CpG island showed 8 cases hypomethylation changes, 17 cases hypermethylation changes, 31 cases no methylation changes. The degree of methylation changes showed a tendency to cluster in a range of U1 and M1 low-grade changes. As a result of the relation between methylation changes and clinicopathologic factors, non-CpG island in several genes mainly showed hypermethylation but CpG island in several genes rarely showed hypermethylation. Furthermore, relation between methylation and lymph node invasion, in the event of lymph node invasion, p16 stream 0.7 kbp, p16 upstream 1.0 kbp, hMLH1 upstream 1.0 kbp showed hypomethylation and BGLAP upstream 4.5 kbp, Runx3 upstream 1.7 kbp, KIAA downstream 0.4 kbp showed hypermethylation but the rest of the genes were not changed.

In 29 tumor foci, a LOH was found most frequently on chromosome 3p, 8p, 9p, 13q. Chromosomal loss and yielded an overall mean value of  $4.79 \pm 2.2$  per tumor focus.

**Conclusions:** The head and neck cancer and its progression generally need the proper level of chromosomal losses were accomplished cancer progression or development but over the level of changed could not affect cancer progression. This study showed that methylation pattern and LOH might be important rules and target event in head and neck cancer. From now on there will be a experiment about finding a point of the genetic modification and find the way to prevent the cancer.

#### 402 POSTER Bioengineering reconstruction of the upper respiratory tracts in rabbits

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**Background:** The Objective of this study is: To perform the reconstruction of the upper respiratory tract using tissue-equivalent. The tissue-equivalent includes cellular culture of fibroblasts and of epidermic keratinocytes attached to the polymer biocompatible net.

**Materials and Methods:** For the reconstruction of the upper respiratory tract was used the bioengineering transplant. Transposed muscular flaps are used as the basis for implantation of stem-cells. Mucous membrane is restored by means of a tissue-equivalent. The tissue-equivalent includes allogeneic cellular culture of fibroblasts and of epidermic keratinocytes attached to the polymer biocompatible net. 10 rabbits were treated by this method.

Results were compared with a control group that for the reconstruction of the defect upper respiratory tract using a only sternocleidomastoid muscle. Wound healing and the epithelization of muscular flap were compared between groups.

**Results:** All reconstructed animals survived the postoperative period. After 2 weeks of the muscular flap was epithelized to 50% in the experimental group of the animals, to 20% in the control group. After 4 weeks muscular flap was completely epithelized in both groups of the animals. A morphological study showed the presence allogeneic tissue-equivalent during 30 days on the muscle after the implantation.

**Conclusion:** The research done covers the fundamental mechanisms of tissue repair and practical aspects of application of tissue cellular transplants. Allogeneic transplant contributes to epithelization and is capable to remain after implantation in the course of 30 days. Protocol of experiment approved by Ethics committee.

#### 403 POSTER Specificity and sensitivity of point mutation detection in tumor suppressor genes via chemical cleavage of mismatches

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Tumor suppressor genes are inactivated by random point mutations the appropriate identification of which is applied for cancer diagnosis, prognosis, monitoring, target chemotherapy. Elaboration of the methods which detect random point mutations of all types and are sensitive enough to reveal mutant DNA in the presence of wild-type DNA represents an actual goal for modern molecular oncology. The most promising approach