

**EXPANSION OF TUMOR-INFILTRATING LYMPHOCYTES FROM HEAD AND NECK SQUAMOUS CELL CARCINOMA TO ASSESS THE SUCCESSFUL EXPANSION FACTORS FOR DEVELOPING ADOPTIVE CELL THERAPY**

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**Background** Adoptive transfer of *in vitro* expanded tumor-infiltrating lymphocytes (TILs) has been effective in regressing several types of malignant tumors, which was first applied to metastatic melanoma more than three decades ago. Promising results of such adoptive cell therapies have intrigued us to evaluate their feasibility in unexplored cancer types that are difficult to treat with existing treatment options. Hence, we evaluated the possibility of TIL expansion from head and neck squamous cell carcinoma (HNSCC), the factors affecting their successful expansion, and the immune phenotypes of expanded TILs.

**Methods** We tried to expand TILs from 51 specimens (36 patients) of surgically resected HNSCC of three different anatomical location groups, including primary tumors and their metastasized lymph nodes (LNs). Cancer tissues were cut into small pieces (1-2 mm each) and underwent initial expansion for 2 weeks with gentamycin (400-1600 µg/ml). The cutoff value of successful expansion was  $0.8 \times 10^5$  TILs per fragment. Factors affecting the successful expansion or contamination were determined mainly by focusing on the location of the samples. The characteristics of expanded cells were evaluated by flow cytometry.

**Results** TILs were successfully expanded from 35% of the samples (18 of 51). Mean number of TILs per fragment in successful cases of LNs was  $6.1 \pm 4.6 \times 10^5$  (n=9), whereas the value was  $4.3 \pm 3.8 \times 10^5$  (n=9) in primary tumors. Among three location groups of the primary tumors, the success rate was higher in samples of oropharynx & larynx than those of oral cavity (44%, 33%, and 19%, respectively). Mean percentage of CD4+ T cells was 60% in compared to 30% for CD8+ T cells. Mean proportion of T<sub>EM</sub>, T<sub>EFF</sub>, T<sub>CM</sub>, and T<sub>NAIVE</sub> were 88.5%, 4.7%, 4.8%, and 1.8% in CD4+ T cells, while 80.0%, 13.4%, 3.6%, and 2.9%, respectively in CD8+ T cells. However, 27% of the total samples were contaminated, and the rate was higher in tumor samples than in LN samples, 36%, and 11%, respectively.

**Conclusions** We could expand TILs from a third of primary tumors and LN samples of HNSCC. LN samples generated slightly more TILs per fragment than primary tumors while getting less contaminated. To use primary HNSCCs as a source of TIL therapy, a setting of clinically applicable antibiotic treatment during the manufacturing process is necessary. Varied success rates were observed according to the location of the cancer tissues, but those remain to be checked statistically with a large number of samples.

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