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T cell repertoire of *ex vivo* expanded tumor infiltrating lymphocytes in patients bearing breast cancer

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In tumor immunotherapy, finding the exact tumor specific sequence of T cell receptor (TCR) in tumor infiltrating T cells (TILs) can assist us in engineering of clonotypes *in vitro*, expand them and finally re-infuse them into patients. In the current study we focused on TCR sequencing data derived from patients with breast cancer using next generation sequencing (NGS). After digesting tumor tissues from nine breast cancer samples, the cells were cultured for two weeks with rapid expansion protocol (REP) media supplemented with 1000 IU/mL IL-2. Then, they were transferred to new media supplemented with 2000 IU/ml IL-2 and 30 ng/ml OKT3 and irradiated healthy peripheral blood mononuclear cells. Afterward, the two week- cultured TILs were analyzed for TCR sequencing via NGS after purification of total RNA. For characterizing the real diversity of TCR sequences between samples, we applied Shannon index. The read count and V/J segments were analyzed by MIX-CR version 1.8.1. NGS data indicated diversity between the TCR repertoires in patients' samples. V/J segment usage in clonotypes was similar within samples but the frequency of each clonotype in samples was various. On average, 1502 clonotypes were observed in TCRβ and 1300 clonotypes were identified in TCRα. Also, we could identify the most frequent V and J segments in TCRβ and TCRα separately in each sample. The more the Shannon index is, the more diverse are the repertoires. Accordingly, in two samples out of nine samples, a high diversity was observed in TCRβ. This is while in the other seven samples, this index was low which is indicative of less heterogeneity. The T cell repertoire in TILs after REP was indicative of heterogeneity of T cells in TCRβ and TCRα.

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