

398 **EXPANSION OF THERAPEUTIC NK CELL LINE UNDER
HYPOXIC AND HYPERBARIC CULTURE CONDITIONS
ENHANCES ANTI-TUMOR POTENCY**

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Background Harnessing NK cells is proving to be an attractive strategy for cancer immunotherapy, either by activating endogenous NK cells or through adoptive cell transfer. NK cells can also be sourced from immortalized cell lines like NK-92 that are currently being evaluated in the clinic. However, the immunosuppressive solid tumor microenvironment (TME) can inhibit NK-92 function thereby limiting its cytolytic properties against tumor cells. To overcome the immunosuppressive effects of the TME, we applied a culturing strategy in which NK-92 cells were serially passaged and expanded under hypoxic and hyperbaric conditions. We then tested the potency of NK cells expanded under TME conditions.

Methods To investigate the short- and long-term effects of TME conditions on NK cell function, we cultured NK-92 cells under several environmental conditions and performed cytotoxic, transcriptional and protein expression analysis. NK-92 cells were either cultured in a conventional CO₂ incubator at 21% O₂ and 0 PSI, or in an AVATAR cell control system which can be precisely tuned to different O₂ and pressure levels that mimic TME conditions. We first assessed the effects of culture conditions on the ability of NK-92 cells to kill tumor cells in both normoxic and TME conditions. To this end we used the AVATAR AI instrument, which employs the environmental control of the AVATAR incubator paired with a specialized plate that enables real-time, label free cell killing analysis via electrical impedance.

Results In normoxic conditions, NK-92 cells that had been exposed to TME conditions for 24 hours exhibited reduced killing, as compared to controls, with slower kinetics of killing and lower total cytolysis. Interestingly, cells that had been adapted to TME conditions for more than 3 months exhibited increased cytotoxic activity, with faster killing kinetics and higher total cytolysis. When cell killing experiments were repeated under TME conditions, the differences between NK-92 culture conditions were even more stark. NK cells grown under standard conditions showed a reduction in total cytolysis, as compared to their killing activity under normoxic conditions. In contrast, TME-conditioned NK cells exhibited robust tumor cytolysis in both normoxic and TME conditions.

Conclusions This initial study supports the hypothesis that NK-92 cells can both react and adapt to different microenvironments. TME-adaptation strategies during cell expansion can enhance potency and efficacy of cell therapies designed to work in a solid tumor microenvironment.

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