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Screening for Immunosuppressive Genes Responsible for Resistance Towards CAR-T Cell Therapy in Cancer Cells

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Abstract

Despite expanding areas of clinical application for CAR-T cell therapy there is still lack of complete information about genes responsible for tumor resistance to this type of therapy. Widely known anti-PD-1 antibody drug Nivolumab and anti-CTLA-4 antibody drug Ipilimumab represent immunotherapeutics that activate the immune system against advanced melanoma and have proven superior to cytotoxic chemotherapy. However, in addition to existing ligands (i.e. CD80, CD86, PD-L1, PD-L2) there are supposedly other surface-expressed ligands stipulating the ability of cancer cells to suppress CAR-T efficacy. It may turn out that tumor resistance to CAR-T therapy is caused by previously unknown immunosuppressive genes. Revealing such genes could improve clinical outcomes by engineering enhanced CAR-T cells and antibodies capable of overcoming the gene-mediated resistance.

In this study we propose the use of CRISPR technique and NGS sequencing to validate this h
and identify novel genes that could presumably determine resistance towards CAR-T cells.

strategy is based on genome-wide screening using human CRISPR/Cas9 Synergistic Activation Mediator (SAM) pooled library that utilize an engineered protein complex for the transcriptional activation of endogenous genes. CAR-T cells were generated from healthy donor T cells genetically modified by lentiviral vectors and expanded *ex vivo*.

Main steps of the experiment include:

1. Amplification of pooled SAM library and use of NGS to confirm library representation.
2. Generation of pooled lentiviral vectors using SAM library and transduction of HeLa cells.
3. Generation of CAR-T cells.
4. Co-cultivation of CAR-T and modified HeLa cells, selection of resistant cells.
5. PCR analysis of sgRNA-containing fragment, followed by cloning of amplicons and NGS of amplicon library.
6. Validation of identified genes to confirm resistance towards CAR-T cells.

We generated a polyclonal CD19-positive HeLa cell line expressing dCas9-VP64 fusion and activation helper protein. This cell line was transduced with Human CRISPR Activation Library (SAM-3 plasmid system) and incubated with anti-CD19 CAR-T cells in order to select resistant cells and identify resistance-determining genes by NGS.

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Disclosures No relevant conflicts of interest to declare.

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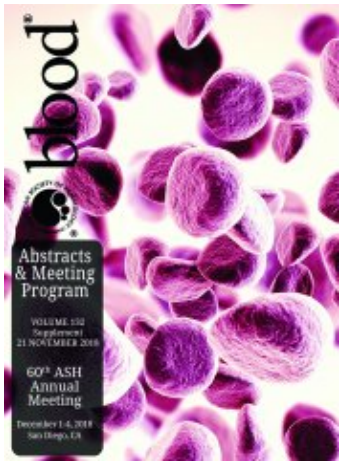

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