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Chimeric antigen receptors in the brain: Can we tackle glioblastoma with engineered NK cells?

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Immunotherapy has been considered a promising treatment strategy for glioblastoma for decades. However, the field has seen a considerable number of disappointing results, particularly with immune checkpoint inhibitors. Adoptive immune cell transfer is a treatment option with a long history but the administration of unmodified or cytokine-stimulated immune cells demonstrated only limited activity or was associated with toxicity.[1] More recently, engineering patient-derived immune cells has become a game changer for hematological neoplasms such as lymphomas or multiple myeloma. Here, T cells that express a chimeric antigen receptor (CAR) have shown powerful antitumor activity, resulting in clinical approval. The situation is more challenging for solid tumors including glioblastoma.[2] Hurdles in this setting include limited access of the administered immune cells to the tumor, leading to poor infiltration, the immunosuppressive tumor microenvironment as well as insufficient persistence of immune cells at the tumor site.[3] A general problem of the currently approved CAR T cell products is the need to isolate T cells from the patient's blood, followed by genetic modification and expansion. While this procedure is feasible, it comes with several limitations in some patients such as an insufficient number of cells isolated, difficulties in the transduction procedure, delays in the manufacturing process, and high cost.

In the current issue of *Neuro-Oncology*, Burger et al. report on the dose-escalation part of the phase 1 CAR2BRAIN study, which addresses some of the issues associated with adoptive immune cell transfer. In this clinical trial, NK cells, engineered to express a HER2-specific CAR, were intratumorally administered to patients with HER2-positive recurrent glioblastoma.[4] The investigators took advantage of a previously generated GMP-compliant immortalized NK-92 single-cell clone (NK-92/5.28.z) that carries a second-generation CAR recognizing the HER2 (ErbB2) antigen. These cells had shown strong therapeutic activity in preclinical models against HER2-positive gliomas. Intracranial injection to glioblastoma patients was safe and the presented results form the basis for further clinical development of local CAR-NK cell therapies.

The NK-92 cell line offers several advantages compared to T cells such as their immediate and almost unrestricted availability not requiring leukapheresis. Administration of NK cells is also possible regardless of the patient's MHC composition. The use of CAR-NK cells may also be associated with less toxicity such as cytokine release syndrome and immune effector cell-associated neurotoxicity syndrome (ICANS) compared to T cell-based therapies.[5] Intra- and intertumor heterogeneity as well as antigen loss have remained important hurdles for targeted immunotherapy as seen for CAR T cell strategies targeting EGFRvIII.[6] HER2 is a well-characterized target for immunotherapy in other cancer types and is also present in a subset of glioblastomas. Of note, it seems to be rather stably expressed, which reduces the risk of immune escape due to antigen loss. However, as only patients with HER2-positive glioblastoma are eligible for the construct used in the present study, the search for antigens that are homogeneously and stably expressed will remain an important research area.

There is a considerable number of additional questions that remain to be addressed. While the current study used an irradiated NK cell line, the clinical arena in hematological malignancies is dominated by approved T cell products. A comparison between different immune effector cells, possibly also taking macrophages into consideration, will be important to define the ideal immune cell subset for genetic engineering and subsequent adoptive transfer. The combination of CAR-based immunotherapy with conventional treatment options such as irradiation may result in synergistic activity and should be investigated in future studies.[7] Furthermore, the antitumor activity and persistence of adoptively transferred immune cells may be further improved by armoring them with activating cytokines.[8] Alternatively, blocking immunosuppressive signals may help boost the activity of CAR-based immune cells, a strategy that is planned for the second part of the CAR2BRAIN study. Larger patient cohorts and additional translational research will be needed to assess if the limited persistence of irradiated NK cells is sufficient to exert clinically meaningful antitumor activity. Furthermore, the identification of novel targets that are ideally (1) exclusively expressed by tumor cells, and (2) not only present in a fraction of glioblastomas, will be crucial to expand the number of patients who are potentially eligible for a CAR-based immunotherapy approach.[9,10] Finally, while intracranial treatment was safe and feasible in the CAR2BRAIN study, treatment was restricted to a single administration of NK cells that was done during surgery. Repeated injections, which

may result in stronger and more sustained therapeutic effects, require catheter placement. While this is planned by the investigators, it needs to be awaited if this approach is still feasible with an acceptable safety profile and without undesired effects on the patient's quality of life. Systemic administration of CAR-based immune cells would be much easier to implement in clinical routine but requires additional research efforts to achieve a sufficient accumulation of immune effector cells at the tumor site without causing systemic toxicity.

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