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POSTER ABSTRACTS

704.CELLULAR IMMUNOTHERAPIES: EARLY PHASE AND INVESTIGATIONAL THERAPIES

Genetic Engineering of Hematopoietic Progenitor Stem Cells for Targeted IFN-α Immunotherapy Reprogramming the Solid Tumor Microenvironment: A First-in-Man Study in Glioblastoma Multiforme (NCT03866109)

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Background and methods. TEM-GBM is a Phase 1/2a dose-escalating non-randomized open label study, involving a single intravenous injection of Temferon. Temferon is constituted by autologous CD34+ HSPCs genetically modified with a lentiviral vector encoding for human interferon-a2 and has been designed to deliver IFNa in the tumor microenvironment (TME) in a targeted manner via Tie-2 expressing monocytes (TEMs). The patient population is newly diagnosed GBM patients with unmethylated MGMT gene promoter. The study aims to evaluate the short-term (up to 90 Days) and long-term (up to 2 years) tolerability and safety of five escalating doses of Temferon in up to 27 glioblastoma patients. The secondary objective is to evaluate biological activity and efficacy when used following SOC first line therapeutic approaches. Autologous CD34+ HSPC are mobilized with lenograstim and plerixafor, collected by apheresis, purified and ex vivo modified with a lentiviral vector. So far, up to 3 million Temferon cells/kg have been co-administered with a fixed dose of non-manipulated CD34+ supporter cells following a sub-myeloablative conditioning regimen (Thiotepa + BCNU or Busulfan or Busulfan alone).
Results. As of 30th June 2023, twenty-one patients have been enrolled across 7 cohorts evaluating 4 incremental doses (0.5-3.0x10 ^{6}/kg) of Temferon. To date, no DLTs have been identified. Median Overall Survival after 1st surgery is 17 months (5-40 months). In all patients, rapid engraftment of gene modified progenitors and fast recovery from sub-myeloablative conditioning regimens have been observed (median engraftment across all the cohorts: Neutrophils D+12, Platelets D+13,5 following Temferon infusion). The percentage of transduced cells found in the BM obtained for the highest dose reached 50% at 1 month and persisted at detectable level in the long-term. Very low median concentrations of IFNa were detected in the plasma, indicating tight regulation of vector expression. Notably, the concentration of IFNa in the CSF increased concomitantly with disease progression suggesting active recruitment of TEMs into the tumor and subsequent release of IFNa. 57% of the treated patients underwent a 2nd-line treatment (either pharmacological or surgical) with an interim survival rate at 2-years of 28% (5 of 18 patients; 3 patients excluded as follow-up is below 12 months), which is higher than what is reported

in literature (15%). One out of the surviving patients was enrolled in a long-term follow-up study and survived up to 3 years after surgery without any 2nd-line therapy.
Genetically engineered cells were detected within the tumor lesion at levels >3% in 3/6 patients at second surgery. Single cell RNA seq analysis unveiled reprogramming of the myeloid TME, with an increase in pro-inflammatory (M1-like) and a decrease in hypoxic (M2-like) macrophages following Temferon exposure. Of note, this population shift was particularly evident when comparing a stable with a progressive tumor lesion biopsied contemporaneously in one patient, reproducing preclinical findings from a GBM mouse model (PMID: 35857642). Analysis of the T cell compartment highlighted an overall increase of CD8+ T cells (mainly effector T cell subsets) and a decrease in CD4+ T cells in Temferon patients. By mapping published signatures of antitumor neoantigen-reactive T cells (NeoTCR; PMID: 35113651) onto the GBM T cell landscape and integrating this data with clonal frequency metrics from concurrent scTCRseq data, we observed an approximately 3-fold increase of predicted tumor-reactive CD8+ T cells bearing expanded clonotypes (>1% within each patient) in Temferon vs. control patients. Strong up-regulation of IFNa and inflammatory responses in most of the clusters from myeloid and T cells, but also CD45- tumor and stromal cell compartments from Temferon patients suggested diffuse IFNa payload delivery into the GBM TME.
Conclusion: These data show that Temferon is safe and biologically active at the tumor site and favors anti-tumor immunity. The results provide initial evidence of Temferon's potential to modulate the TME of GBM patients and to counteract disease progression and improve the survival of uMGMT GBM patients.

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