Background The dynamic regulation of the tumor microenvironment (TME) is a key mechanism driving clinical outcome with immunotherapies. The presence of different macrophage phenotypes in the TME may either promote tumor growth (M2) or phagocytose cancer cells and remodel the TME (M1). Influencing macrophage polarization may be an important target for immunotherapy.

Methods Temferon is an autologous HSC-based platform that delivers targeted IFNα into the TME via Tie-2 expressing monocytes, with transcriptional & post-transcriptional control mediated by miRNA target sequences. Temferon is being administered in a Phase 1/2a clinical trial in 21 newly diagnosed glioblastoma (GBM) patients with unmethylated MGMT promoter.

Results As of July 2022, 4 escalating doses of Temferon (0.5-3.0x10⁶/kg) were tested across 17 patients assigned to 6 cohorts. Current total follow-up from Temferon infusion is 7–749 days (3–28 months after 1st Surgery). Seven recurrent tumors were resected and where enough fresh material was available gene-marked cells were identified in the CD45+ tumor infiltrate in 2/2 specimens (3%-5%). scRNA analysis of the myeloid TME compartment (n=4 Temferon patients) detected a broad induction of an IFN, TNF/NFkB and hypoxia response relative to n=6 standard-of-care treated patients and unveiled an overrepresentation of pro-inflammatory macrophage clusters. Strikingly, this closely resembled the microenvironmental changes observed in a murine GBM model treated with IFN gene therapy (Birocchi, Sci Transl Med, 2022), where a shift in the M2 to M1 macrophage balance was associated with tumor responses. Notably, the highest proportion of pro-inflammatory macrophages was detected in a stable lesion biopsied from a patient that had a contemporaneous progressing lesion, which instead contained the lowest pro-inflammatory macrophage quantity. Analysis of the T cell compartment (scRNAseq+TCRseq) of the stable lesion highlights the presence of specific CD8+ T cells clones characterized by an effector phenotype with an inflammatory profile (IFNa/g response, TNFa signaling).

Conclusions These data support the hypothesis that Temferon, by acting on the M1-M2 balance, favors a pro-inflammatory state that, as predicted by preclinical studies, induces an immune system reset that may favor containment of GBM growth.

Ethics Approval The TEM-GBM Clinical Trial (NCT03866109) has been reviewed and approved by the Italian Competent Regulatory Authority, AIFA, on 26/09/2018. The Coordinating Etichal Committee, San Raffaele Hospital (Milan), approved the study on 12/07/2018 and then on 11/10/2018.