

P3.171: Longitudinal Analysis of Evolving CSF Microenvironment after Intrathecal Pemetrexed in EGFR-mutated Patients with Leptomeningeal Metastases

Meng Qiao¹, Ronghui Yan², Yichun Zhu¹, Xiaodan Zhu¹, Jie Liu¹, Yong Zhang¹, Yanhui Xu², Jie Hu¹, Yuanlin Song¹

¹Department of Pulmonary and Critical Care Medicine, Zhongshan Hospital, Fudan University, Shanghai, China. ²Fudan University Shanghai Cancer Center, Institutes of Biomedical Sciences, Shanghai Medical College of Fudan University, Shanghai, China.

Correspondence: Yuanlin Song, yuanlin.song@zs-hospital.sh.cn

INTRODUCTION

Leptomeningeal metastasis (LM) represents a devastating complication of non-small cell lung cancer (NSCLC) with poor prognosis and limited therapeutic options. Currently, intrathecal pemetrexed (IP) has emerged as a potentially viable treatment strategy for LM patients, yet how sequential IP reshapes cerebrospinal fluid (CSF) tumor cells and the immune microenvironment remains unclear.

METHODS

- Two EGFR-mutated patients with cytologically confirmed LM were enrolled in this study.
- Comprehensive CSF analysis of 7 samples at different timepoints obtained from Ommaya reservoir via single-cell RNA sequencing (SeekOne DD single-cell 5' Transcriptome Kit, SeekGene Biosciences) was performed.

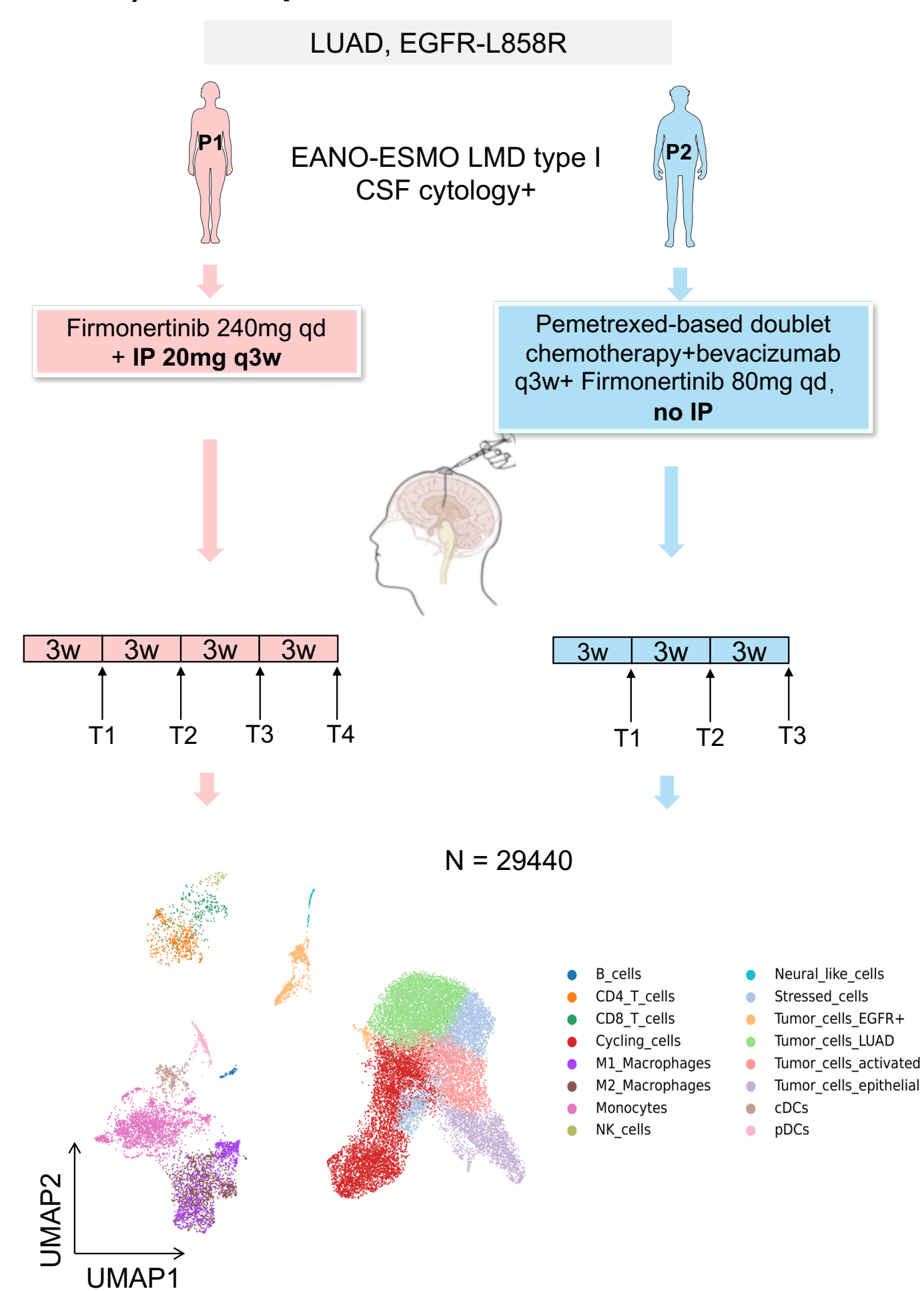


Figure 1. Schematic overview of this study

DISCUSSION AND CONCLUSION

Take Away Message

- Tumor cells in CSF acquired CNS lineage plasticity upon IP treatment.
- Upregulation of HSP90AA1, GDF15 and MT-ND3 facilitated protein stabilization, metabolic reprogramming and immune escape following IP treatment.
- Intensive crosstalk between GDF15⁺ tumor cells and immune cells induced CD8⁺ T cell anergy and M2 macrophage polarization, hence formed immunosuppressive microenvironment.

Future Directions

- Consistent with the principle that **“what does not kill me makes me stronger”**, chemotherapy pressure selects more aggressive and immunosuppressive tumor subclones. Furthermore, repeated intrathecal treatment induces CD8⁺ T cell exhaustion and M2 macrophage polarization, ultimately forming an immunosuppressive CSF niche.
- We aim to propose that **future LM studies should focus not only on survival outcomes induced by pemetrexed but also on treatment-induced CSF microenvironmental remodeling**, providing novel insights for developing rational intrathecal chemo-immunotherapy combination strategies.

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Artificial Intelligence (AI) Use Disclaimer

The authors declare that no AI tools have been employed in any stage of this work. All intellectual contributions, text, figures and conclusions are original from authors.

RESULTS

- Compared with systemic therapy, IP dramatically decreased the tumor cell proportions from 16.4% to 2.4%, particularly depleting cycling cells owing to the cytotoxic nature of chemotherapy

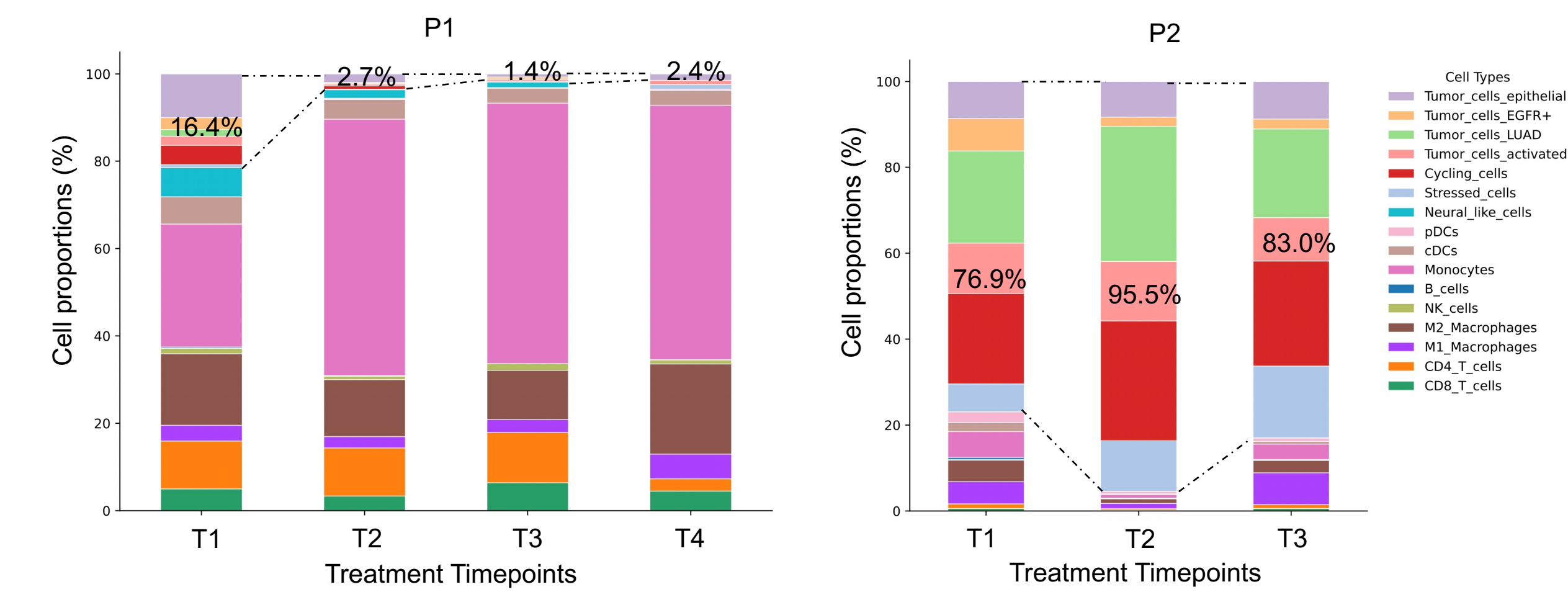


Figure 2. Tumor proportions at different timepoints. Left, P1 with IP treatment; Right, P2 without IP treatment

- Upon IP treatment:** T1: high expression of SFTPB and GLS (origin nature of lung cancer cells) → T2-T3: with high expression of HSP90AA1(enabled tumor cells to fold and stabilize mutated or damaged proteins) → T4: expression of GDF15, MT-ND3 and loss of epithelial lineage marker genes
- No IP treatment:** No obvious evolution of tumor cells

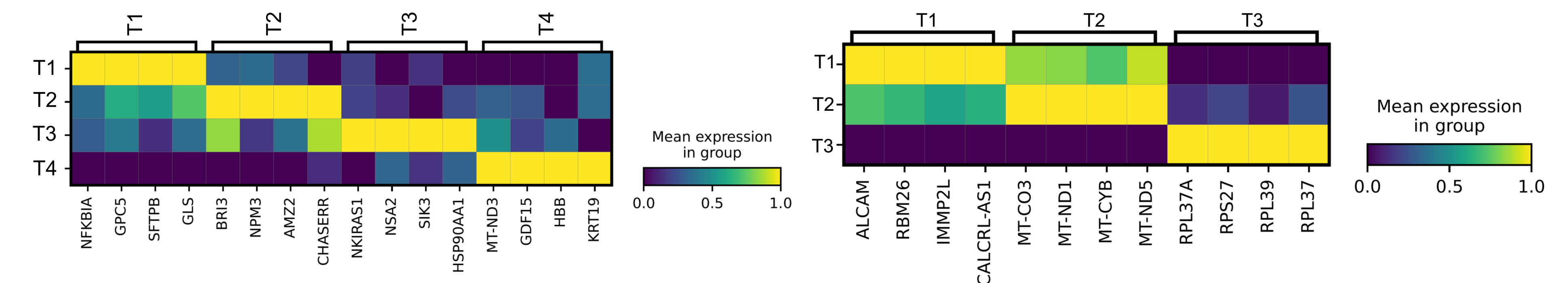


Figure 3. Differential genes expression at different timepoints using NMF. Left, P1 with IP treatment; Right, P2 without IP treatment

- Upon IP treatment, residual tumor cells in CSF acquired lineage plasticity. These cells exhibited significant enrichment in **CNS-specific developmental pathways and morphological remodeling-related pathways**
- Upon IP treatment: 1) induction of CD8⁺ T cell anergy and a striking elevation in M2 macrophage polarization-related gene signatures; 2) the most prominent crosstalk **was between GDF15+tumor cells, CD8+T cells and M2-macrophages**

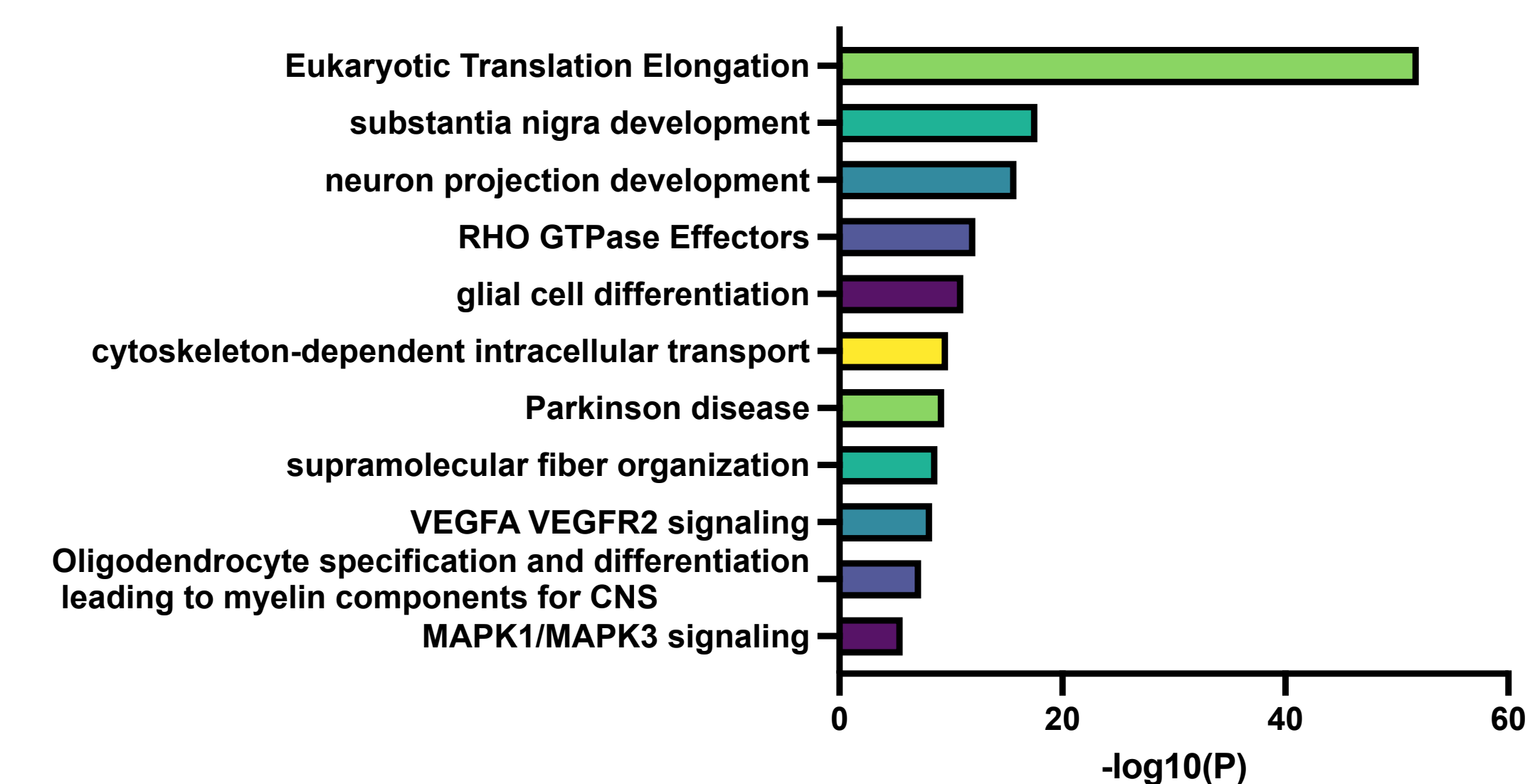


Figure 4. Pathway enrichment analysis on residual tumor cells in T4 sample

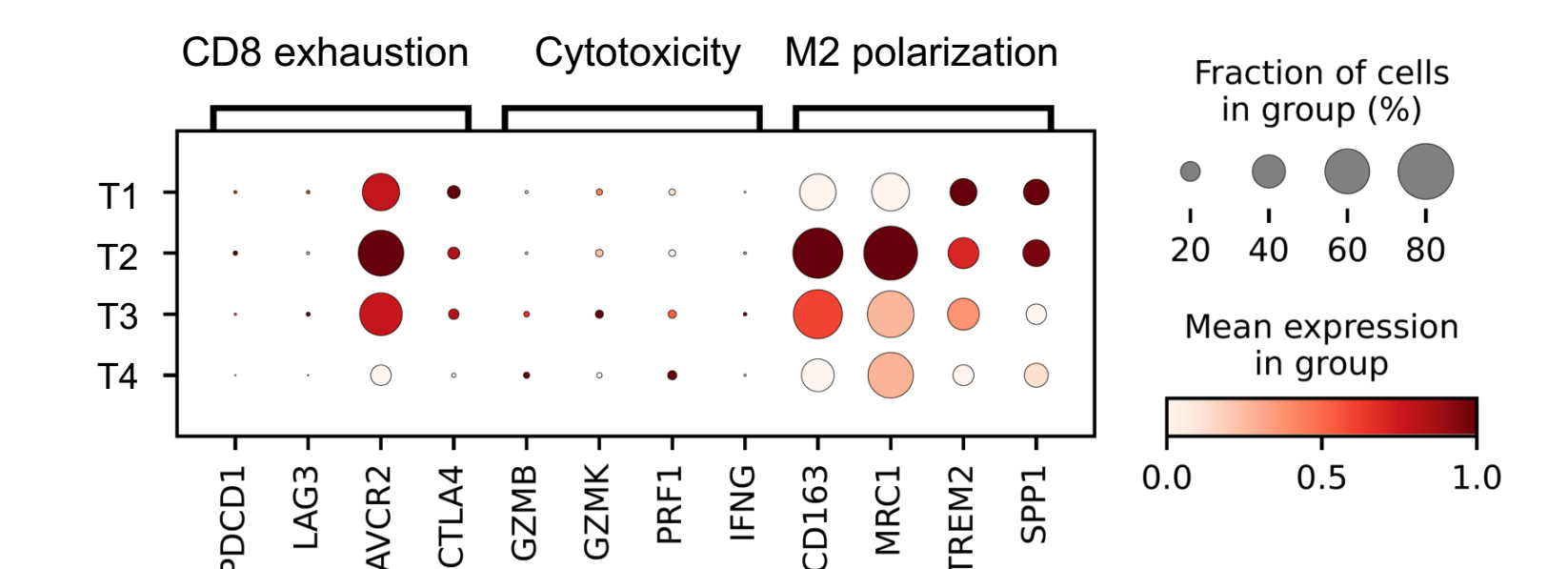


Figure 5. Dynamic profiling of immune-related genes upon IP treatment

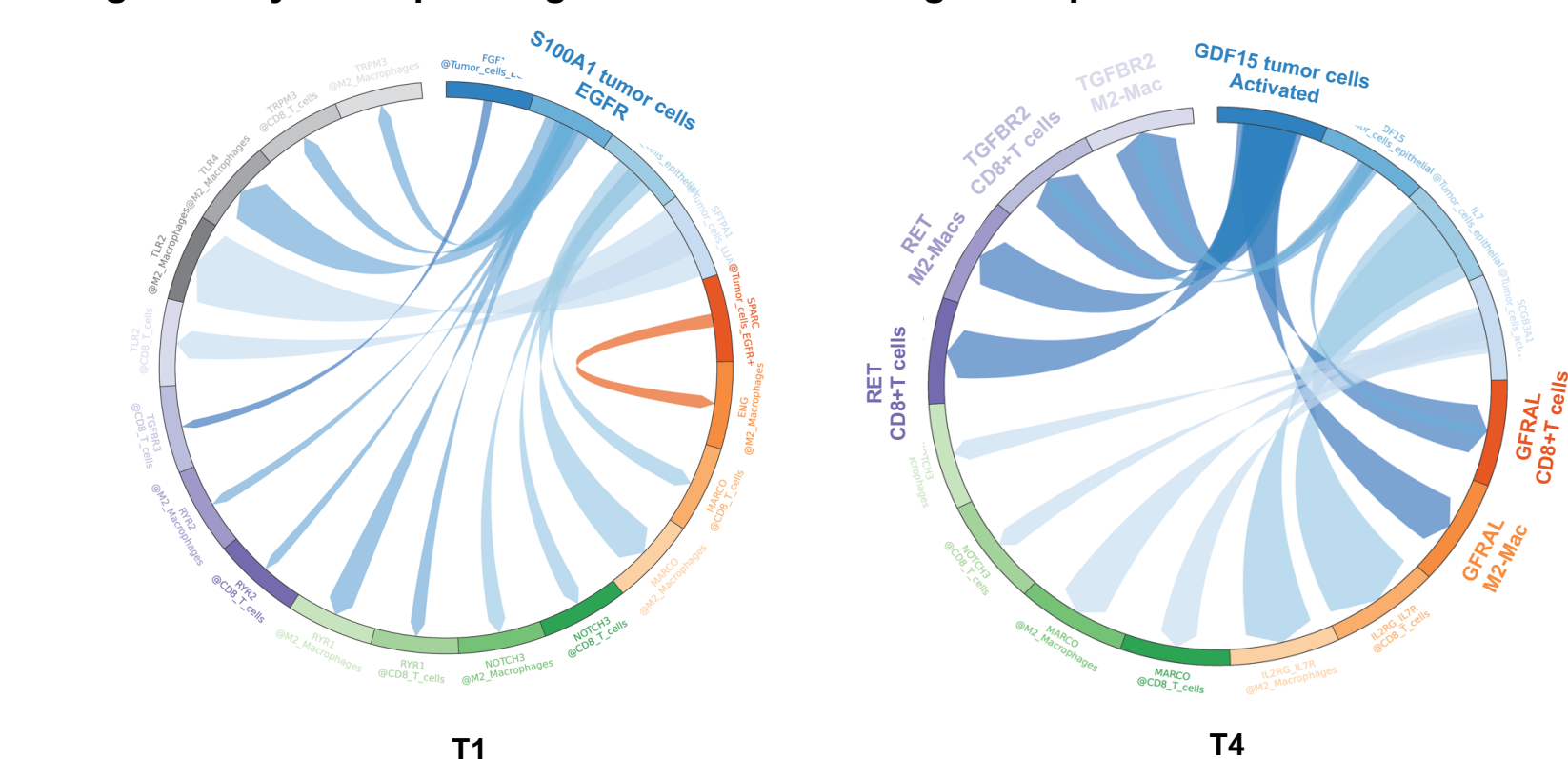


Figure 6. Cellular communication network in CSF using potential receptor-ligand pair interactions