

PHASE 2 TRIAL OF BOTENSILIMAB (Fc-ENHANCED ANTI–CTLA-4), BALSTILIMAB (ANTI–PD-1) AND DOXORUBICIN DELIVERED WITH ULTRASOUND/MICROBUBBLE-BASED BLOOD-BRAIN BARRIER OPENING IN NEWLY DIAGNOSED MGMT UN-METHYLATED GLIOBLASTOMA: INITIAL REPORT OF FEASIBILITY, SAFETY AND IMMUNE CORRELATIVES

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1 Purpose & Rationale

To evaluate the safety and anti-tumoral immunity of botensilimab (BOT, an Fc-enhanced anti–CTLA-4 antibody) and balstilimab (BAL, anti–PD-1) with liposomal doxorubicin (DOX), delivered using low-intensity pulsed ultrasound with microbubbles (LIPU/MB). The rationale integrates three established mechanisms:

I

LIPU/MB — blood-brain barrier opening
Enhances BBB penetration of therapeutics including drugs and antibodies into the brain, and induces immune modulation by microglia activation and eliciting chemotactic signals.^{1,2,3,4}

II

BOT + BAL — immune priming
Demonstrates clinical activity in other immune-resistant cancers and in preclinical glioma models by enhancing T-cell priming, intratumoral Treg depletion and myeloid activation.^{4,5,6}

III

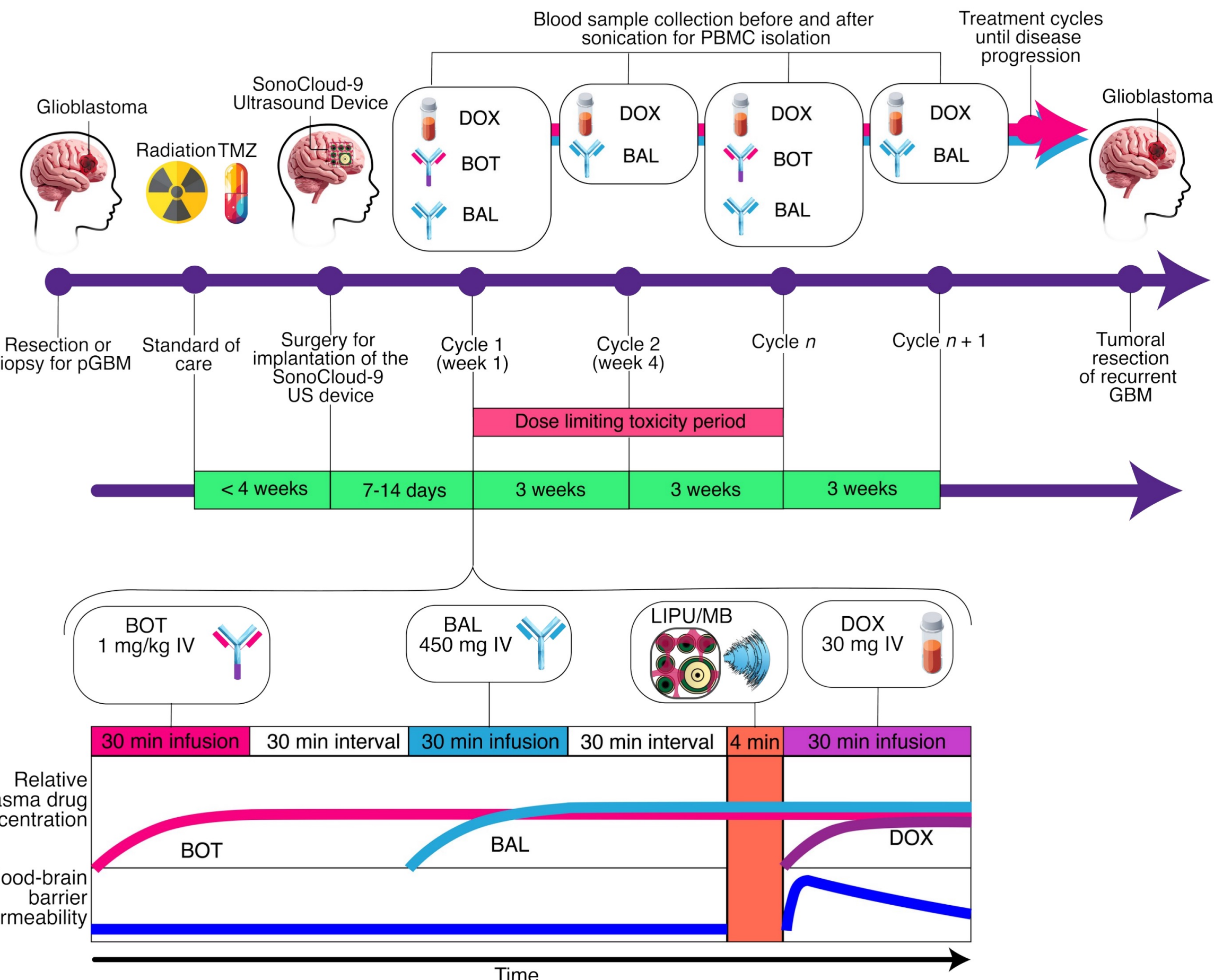
DOX — immunogenic chemotherapy
Enhances anti-tumoral immunity, the expression of FcγRIIIA, a receptor that is critical for the activity of BOT in human glioblastoma microglia, and the efficacy of immune-checkpoint blockade in preclinical models and in human glioblastoma.^{2,4}

2 Methods

Single-arm phase 2a trial. Following radiation therapy, newly diagnosed MGMT-unmethylated glioblastoma patients underwent enrollment and ambulatory placement of an implantable ultrasound device (SonoCloud-9, Carthera, France). Treatment consisted of repeated cycles of LIPU/MB-based BBB opening using the SonoCloud-9, with intravenous BOT/BAL/DOX Q3W (BOT in alternate cycles, up to 4 BOT doses). Adjuvant temozolomide was withheld.

BOT 1 mg/kg IV	BAL 450 mg IV	DOX 30 mg IV	LIPU/MB 4 min sonication
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Trial schema and pharmacokinetic rationale



Treatment timeline from resection through recurrence, and the intra-cycle infusion sequence. BOT and BAL are infused before sonication; DOX is infused immediately after LIPU/MB-mediated BBB opening.

ClinicalTrials.gov Identifier: NCT05864534

3 Feasibility & Safety

12

patients enrolled and implanted

64

treatment cycles administered

4.5

median cycles/patient (range 3–14)

0

implantation complications

✓

All 12 patients completed SonoCloud-9 implantation via outpatient surgery without complications.

✓

64 cycles delivered; median 4.5 cycles per patient (range 3–14).

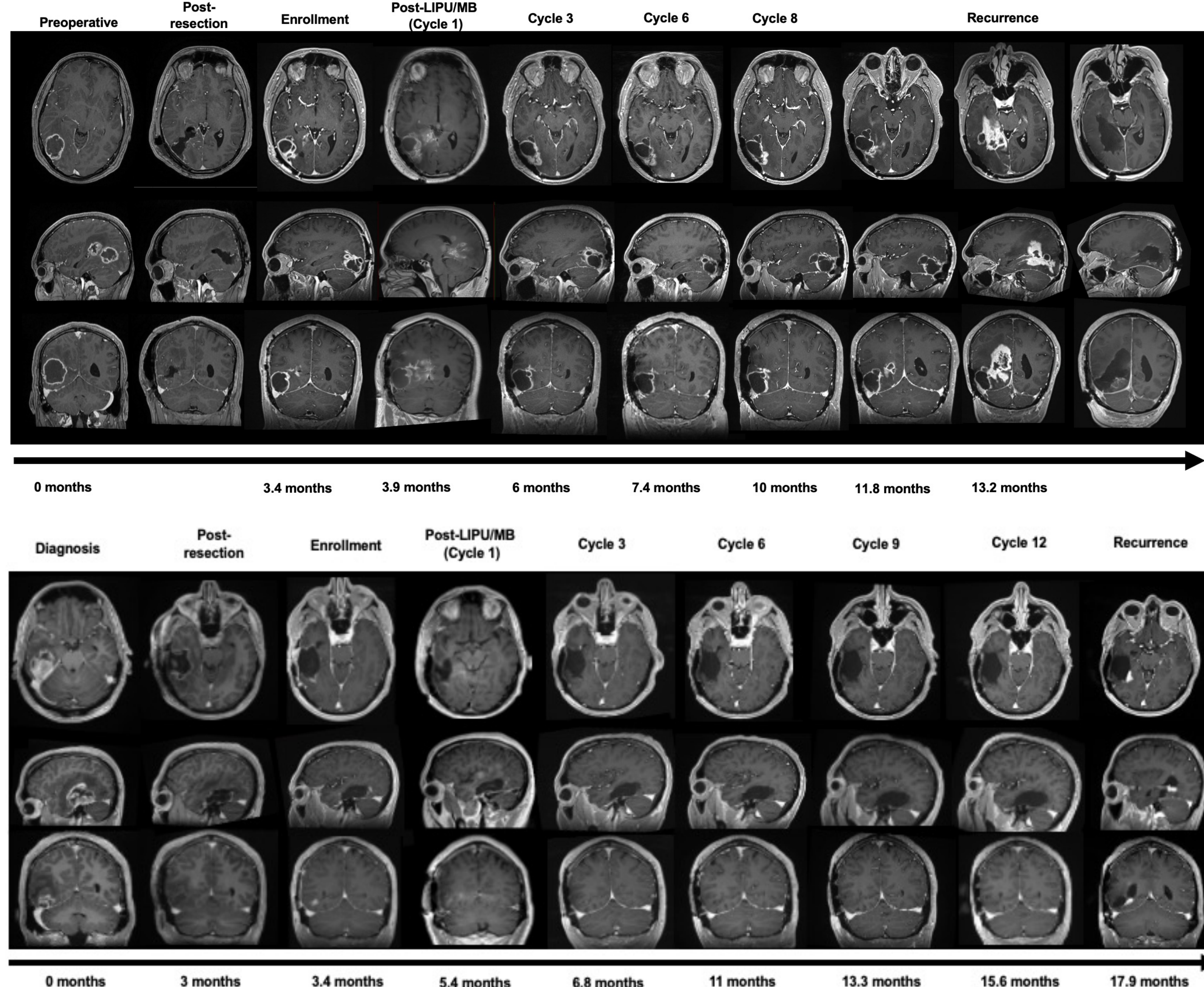
✓

Immunotherapy-related toxicity was as expected for this combination regimen of immune checkpoint inhibitors.

✓

No unexpected treatment-emergent serious CNS adverse events were observed.

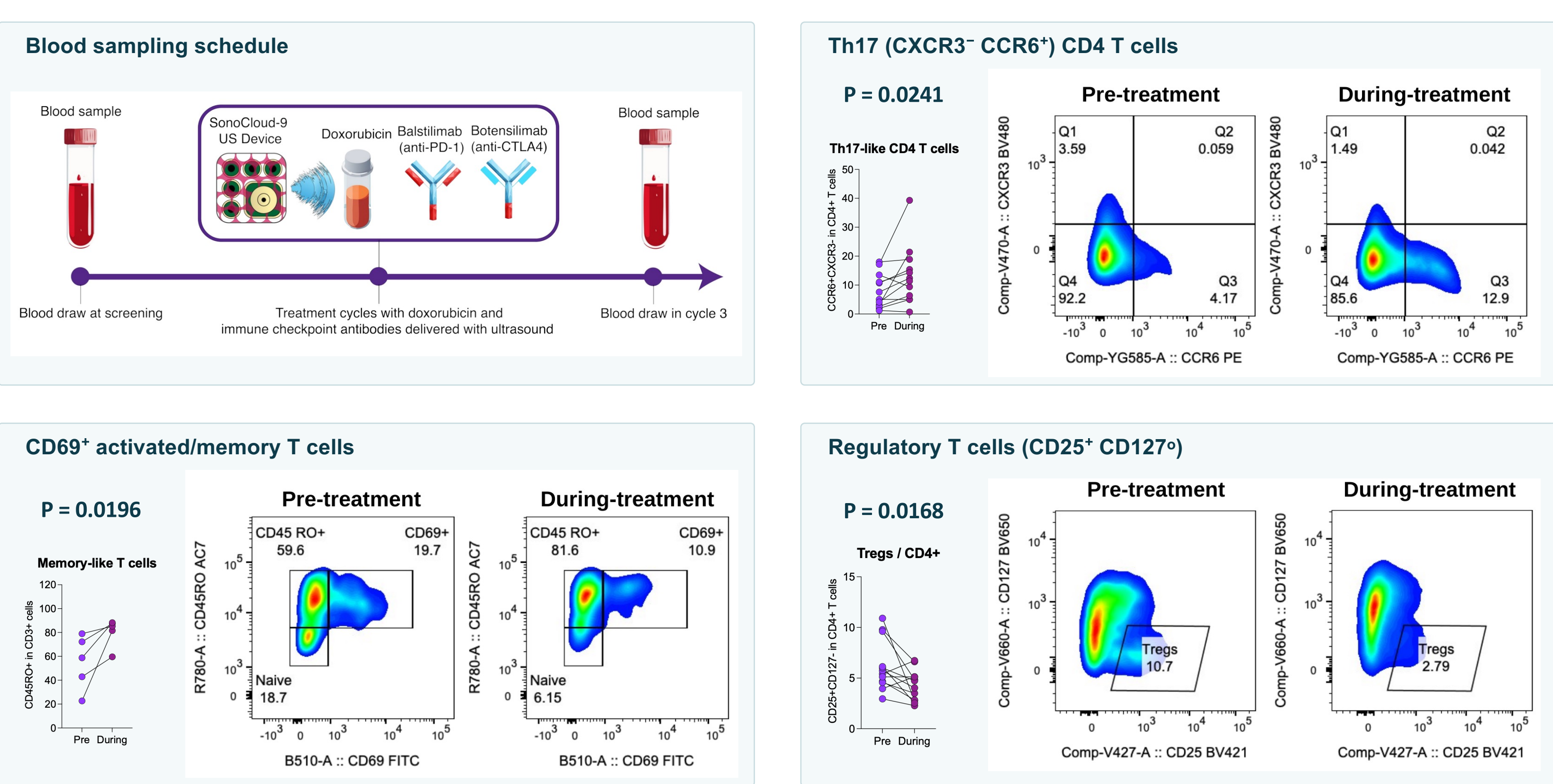
Patients that completed 4 cycles of BOT.



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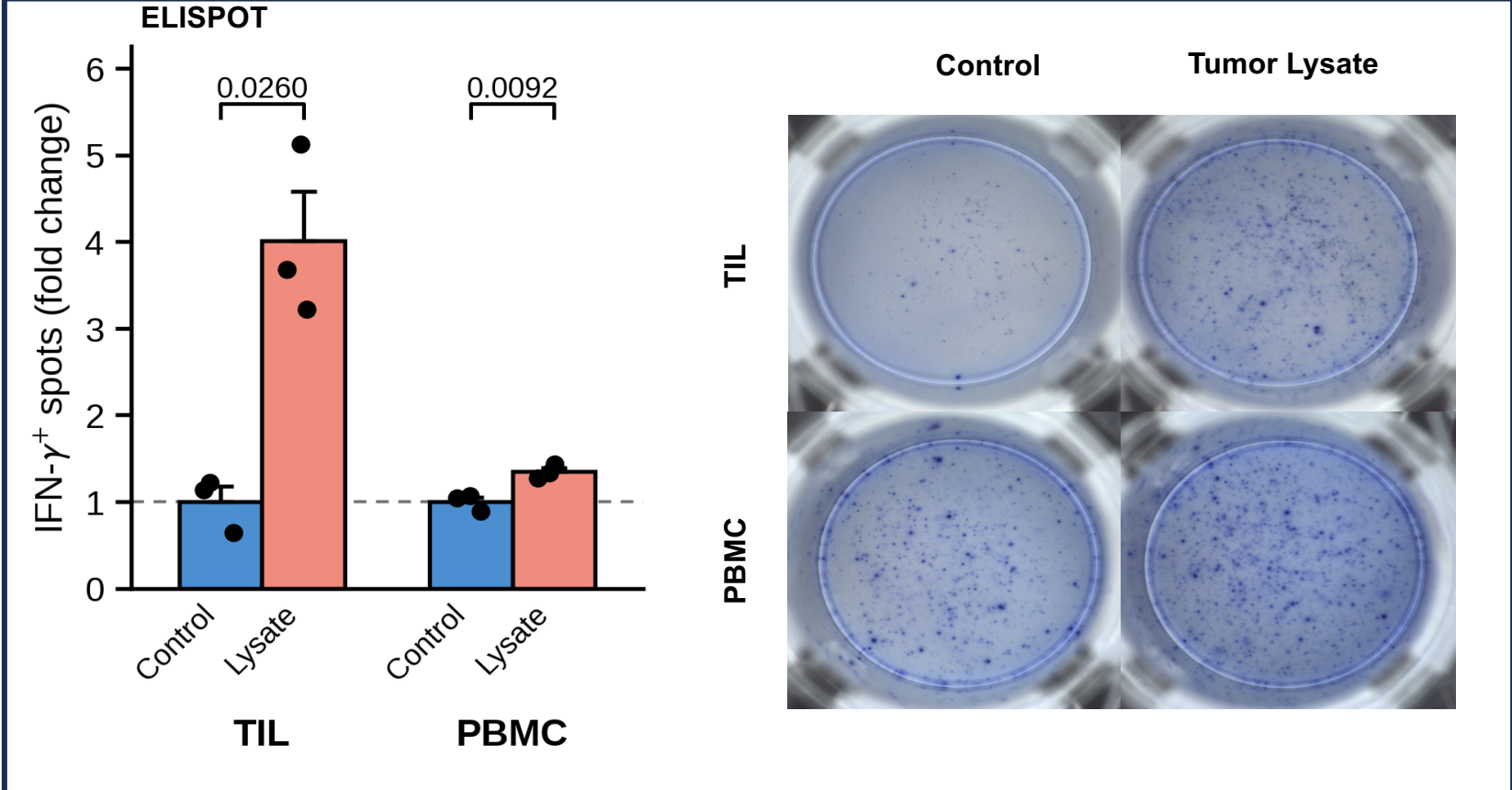
4 Peripheral Immune Pharmacodynamics



Paired pre-treatment and on-treatment (cycle 3) peripheral blood. Treatment was associated with activation of memory-like T-cells, Treg depletion and an increase in Th17 CD4⁺ T cells.

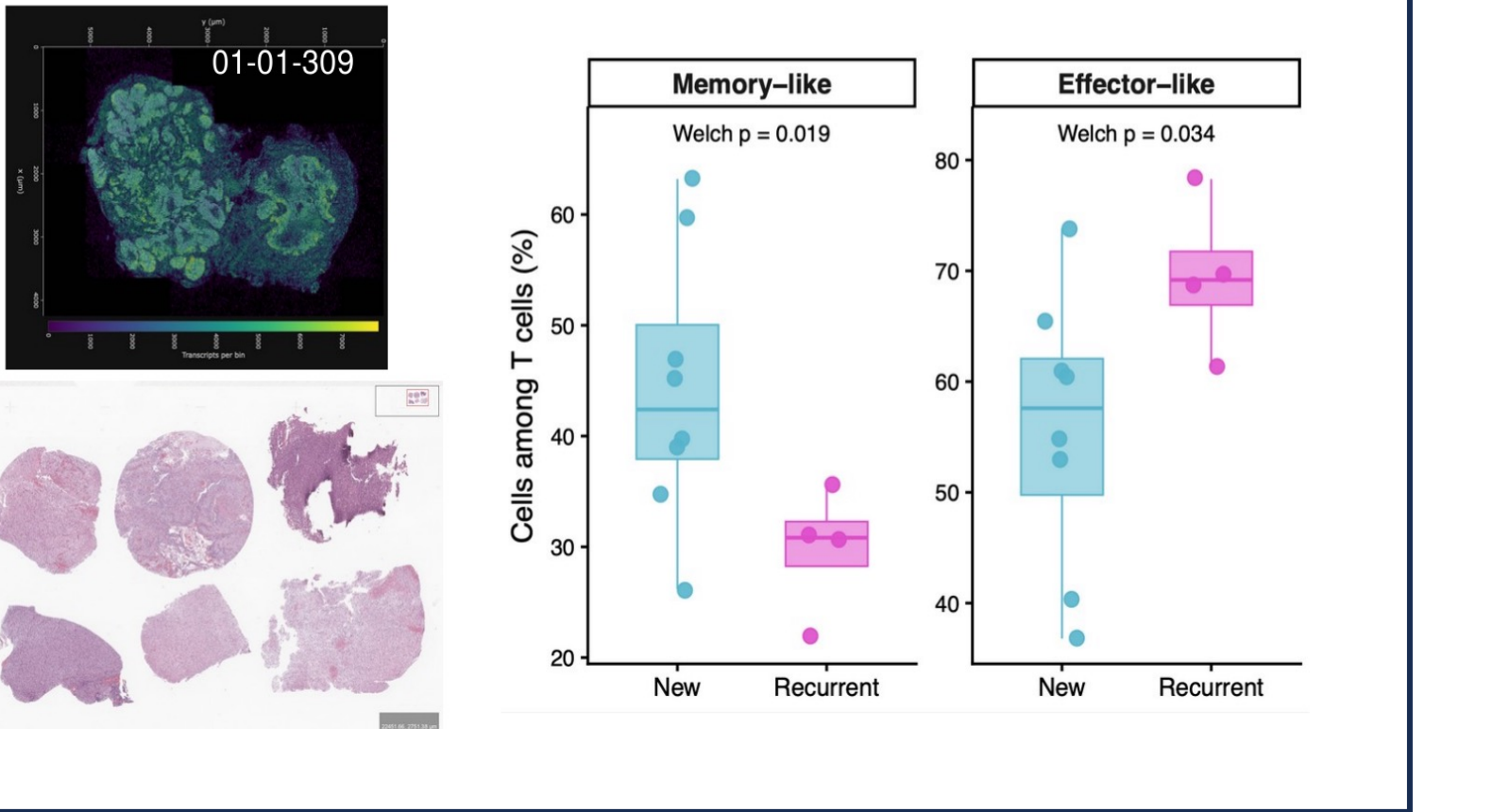
5 Anti-tumor Immunity at Recurrence

ITILs vs PBMC IFNγ ELISPOT against autologous tumor lysate



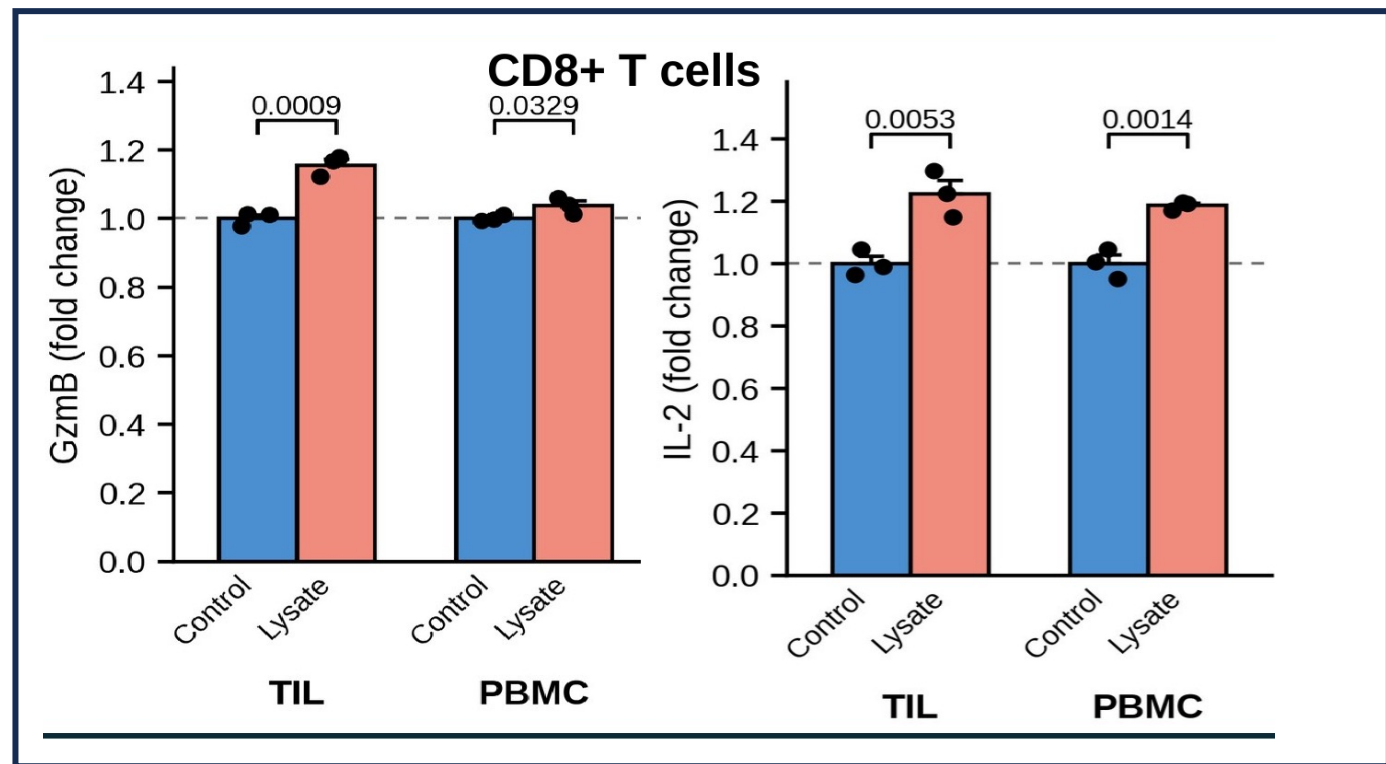
Tumor-infiltrating lymphocytes (TIL) and PBMC from matched samples at recurrence, exposed to control medium or autologous tumor lysate.

T-cell phenotype by spatial transcriptomics



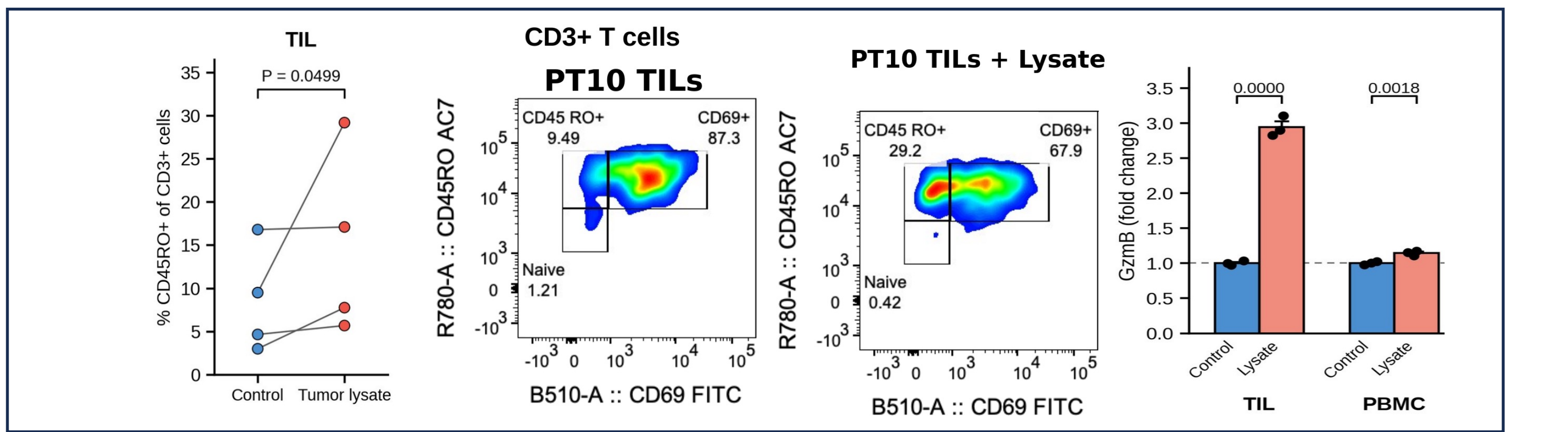
T-cell populations that changed in their proportion at time of diagnosis versus at recurrence following immunotherapy

TILs vs PBMC cytokine responses to tumor lysate



Flow cytometry of TIL and PBMC after tumor-lysate exposure. Granzyme B and IL-2 are upregulated in CD8⁺ T cells (left); IL-2 and IL-17 are upregulated in CD4⁺ T cells (right).

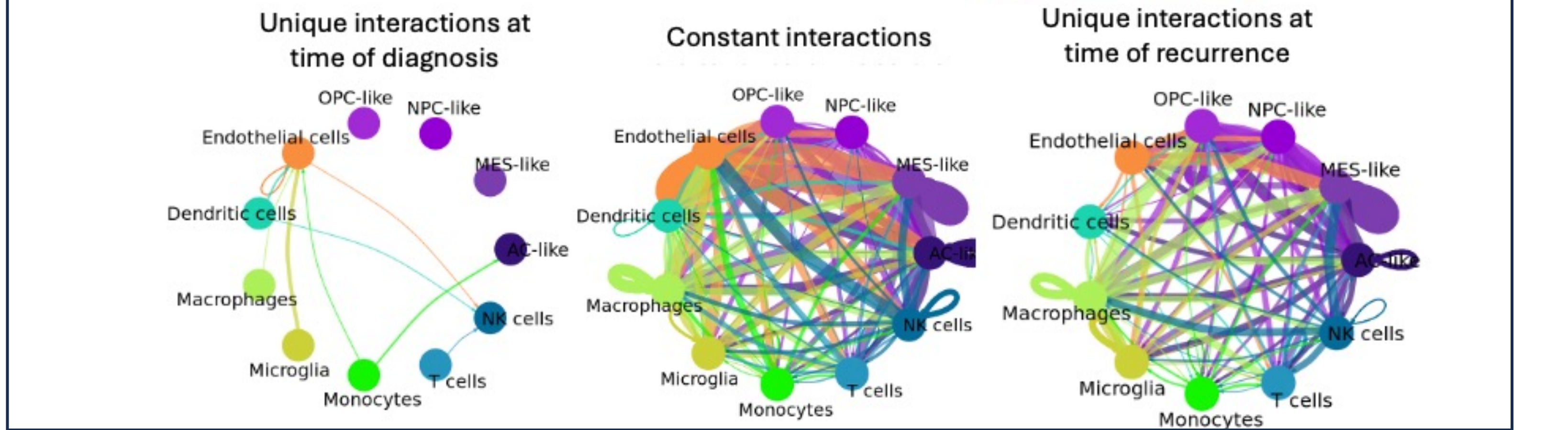
TILs response to lysate



TIL activation after autologous tumor-lysate exposure (left), CD45RO/CD69 activation phenotype of PT10 TILs with and without lysate (centre), and Granzyme B induction in TIL and PBMC (right).

6 Tumor Microenvironment at Recurrence

CellChat interaction networks by spatial transcriptomics on tumor tissue at time of diagnosis versus recurrence



Ligand–receptor interactions inferred from the same data — recurrent tumors show an expanded, densely inter-connected macrophage/microglia compartment engaging tumor cells and T cells.

7 Conclusions

- Combinatorial immunotherapy with LIPU/MB-based BBB opening is feasible in newly diagnosed MGMT-unmethylated glioblastoma, with preliminary evidence of good CNS tolerance.
- Initial immune correlates support T-cell activation to autologous tumor lysate following therapy, with Treg depletion, Th17 expansion and effector cytokine induction.
- Recurrent tumors show emergence of a tumor-associated macrophage infiltrate that is highly inter-connected with tumor cells and T cells.
- Cohort expansion and comprehensive immune profiling will be required to establish efficacy and fully characterize the anti-tumor immune responses induced.

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