Artificial intelligence for effective treatment selection using biodegradable poly(N-(2-hydroxypropyl) methacrylamide) for blood brain barrier delivery and Immune therapy of brain primary central nervous system lymphoma

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Primary Central Nervous System Lymphoma

Approximately 95% of **PCNSL** are diffuse large B-cell lymphomas that are typically highly infiltrative neoplasms characterized as a "whole brain disease," particularly at relapse. Like malignant gliomas, **PCNSL** is not amenable to curative resection. Incidence is increasing for unknown reasons especially in men over 65 years old.

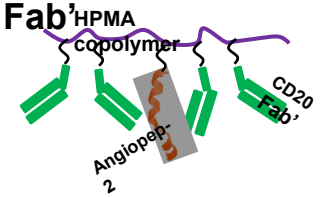
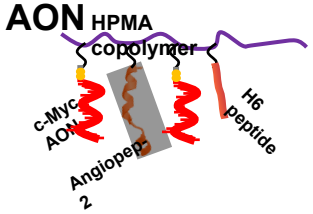
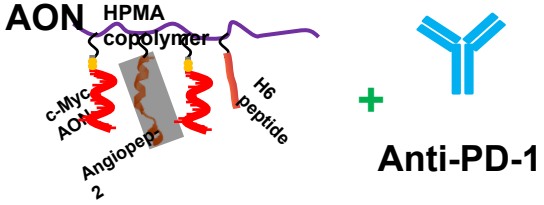
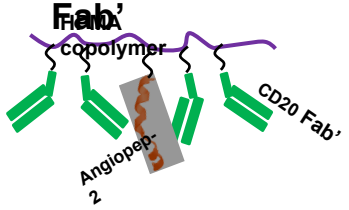
For treated lymphomas located differently from PCNSL, the 5-year survival is **67-79%** (high-dose of methotrexate and radiation therapy or rituximab). However, PCNSL patients that receive treatment have 5-year survival rate of only **20-25%**.

The survival of the elderly patients with **PCNSL** has not changed in 40 years and remains poor at 6 months. At present, there is no standard treatment for recurrent PCNSL.

Multimodal neuro-nanotechnology drug to treat PCNSL tumor

We designed a novel treatment for PCNS lymphoma with multi-functional nano biopolymeric drug based on **poly(*N*-(2-hydroxypropyl))methacrylamide) (PHPMA) nanoplatform**.

- PHPMA-based nano immunoconjugates developed by Kopeček's group are water soluble, non-immunogenic, and biocompatible linear polymers. The second generation of PHPMA copolymer carrier used in this study is biodegradable – it contains enzymatically degradable sequences in the main chain.
- *Importantly, multiple molecules for targeting can be easily attached covalently to PHPMA molecule.* To date, Ab, Ab fragments, peptides, chemotherapeutic drugs, and antisense oligos (AONs) have been successfully conjugated onto PHPMA.

Treatment Design	Drug Action
1. PBS	Control
2. HPMA/AP2/CD20 Fab' 	AP2 peptide-mediated BBB crossing; apoptosis of cancer cells caused by CD20 crosslinking.
3. HPMA/AP2/H6/c-Myc AON 	AP2 peptide-mediated BBB crossing and down-regulation of c-Myc expression in cancer cells by AON.
4. HPMA/AP2/H6/c-Myc AON  + Anti-PD-1	AP2 peptide-mediated BBB crossing; down-regulation of c-Myc oncogene by AON; activation of immune function.
5. HPMA/AP2/CD20 Fab'  + Anti-PD-1	AP2 peptide-mediated BBB crossing; apoptosis of cancer cells caused by CD20 crosslinking; activation of immune function.

Rationale for PCNSL therapy design

New nano immunoconjugates:

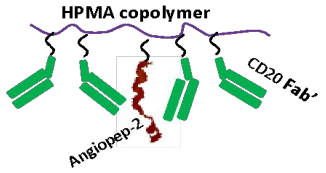
1. cross blood brain barrier (BBB) by attached Angiopep-2 (AP2) peptide*;
2. deliver previously undeliverable checkpoint inhibitor anti-PD-1 to brain tumors, activate local brain immunity
3. to combine immuno and targetable therapy:
   a) c-Myc inhibition and CD20 crosslinking;
   b) c-Myc inhibition and PD-1 receptor blockage;
   c) treatment combination of a, b

*LDLR-related protein (LRP)-1, a member of LDLR family, was evaluated for mediating transport of ligands, such as secreted amyloid precursor protein (APP), across the BBB

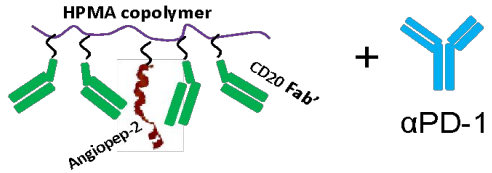
Survival advantage conferred by PHPMA-based nanodrugs with or without checkpoint inhibition in a syngeneic brain lymphoma model (A20 mouse cells)

A. NIC variants

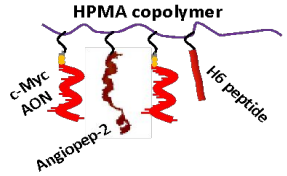
1. HPMA/AP2/CD20 Fab'



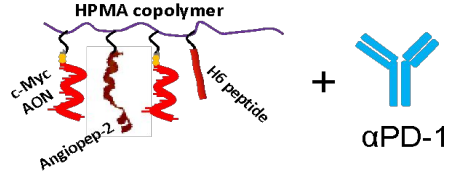
2. HPMA/AP2/CD20 Fab'



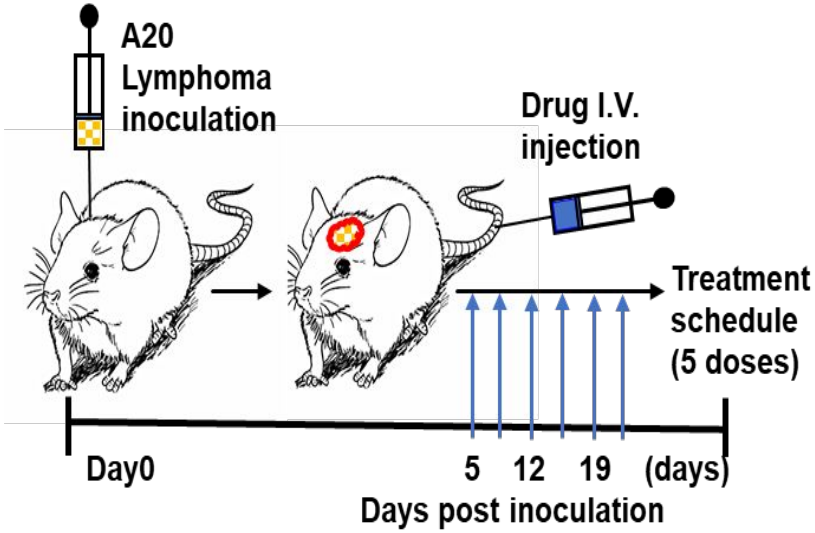
3. HPMA/AP2/H6/c-Myc AON



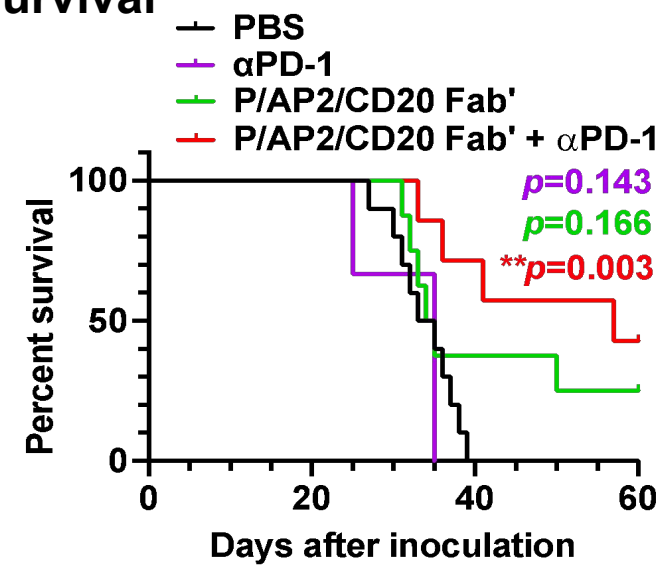
4. HPMA/AP2/H6/c-Myc AON



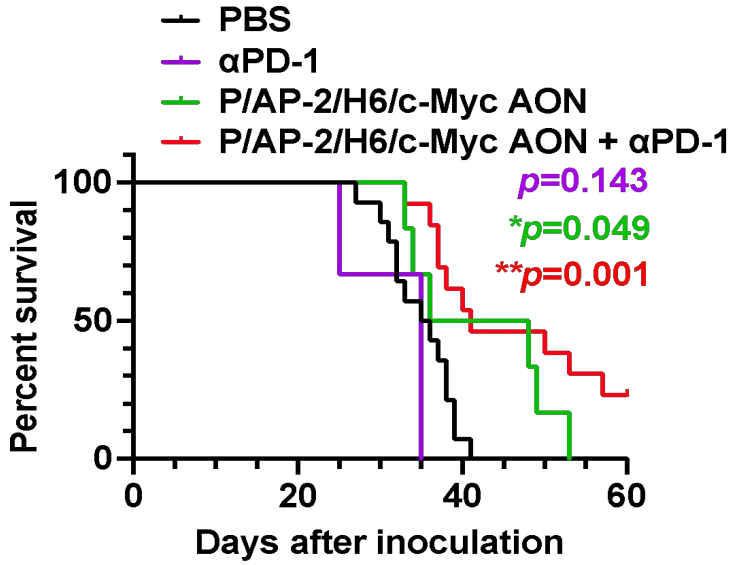
B. NIC administration



C. Survival



**p=0.007 vs. αPD-1

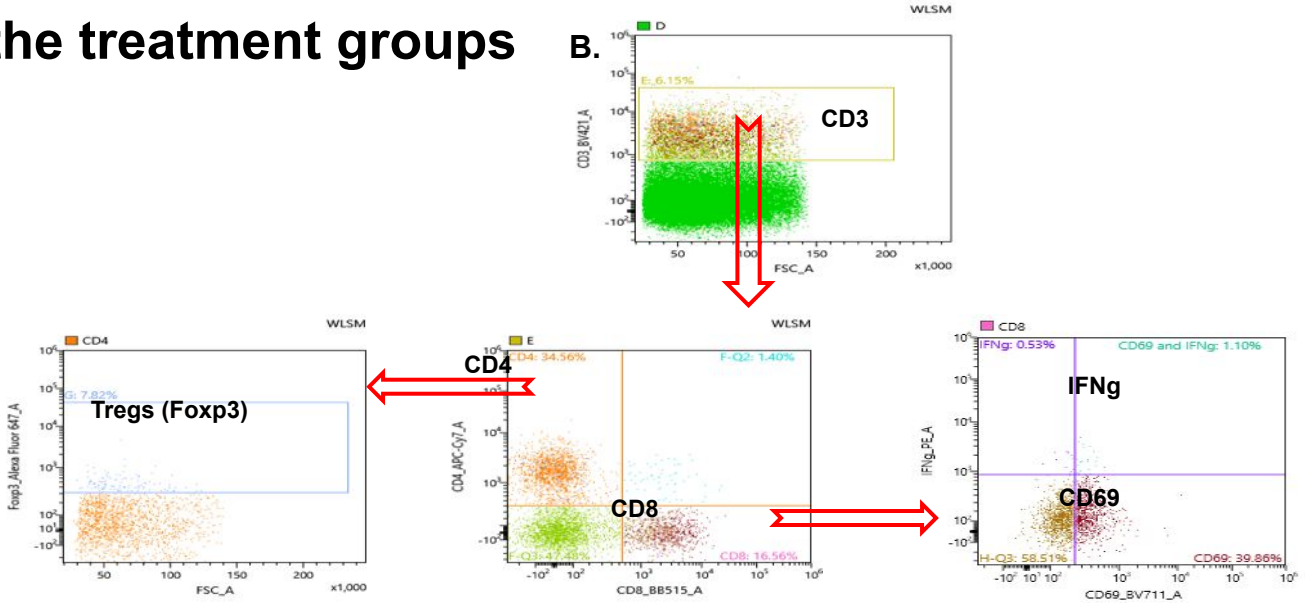


***p=0.0001 vs. αPD-1

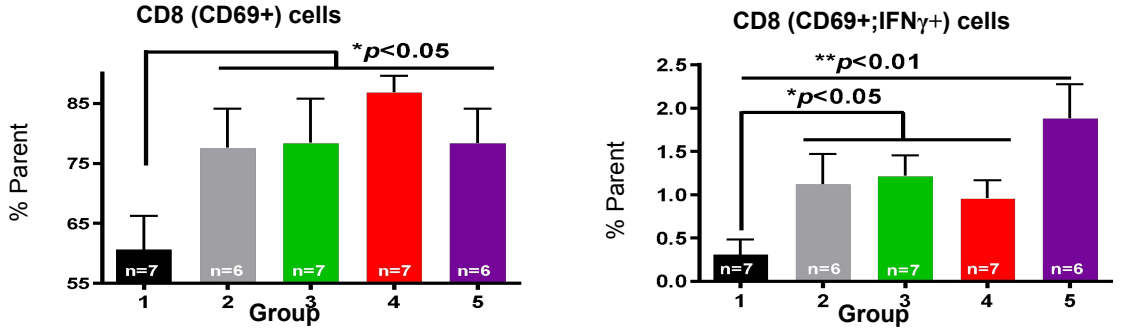
Flow Cytometry Analysis of the treatment groups

Group	Treatment
1	PBS
2	P/AP-2/CD-20 Fab’
3	P/AP-2/H6/cMyc AON
4	P/AP-2/H6/cMyc AON+αPD-1
5	P/AP-2/CD20 Fab’+αPD-1

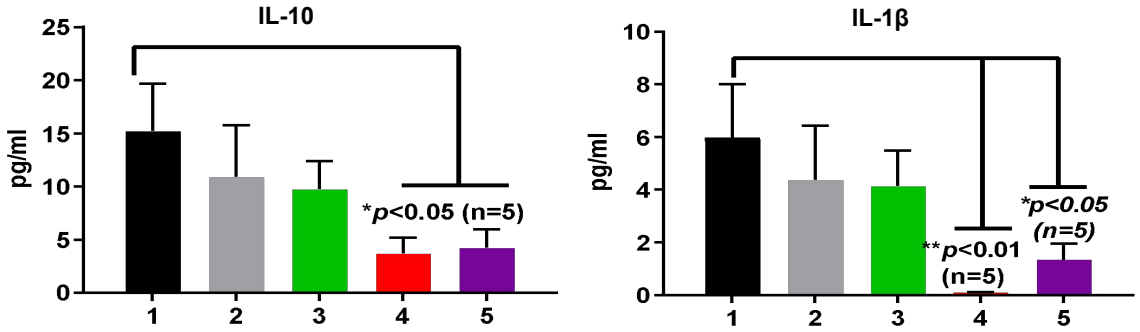
B.



C. Spectral flowcytometry analysis of T cells



D. Cytokine levels in serum

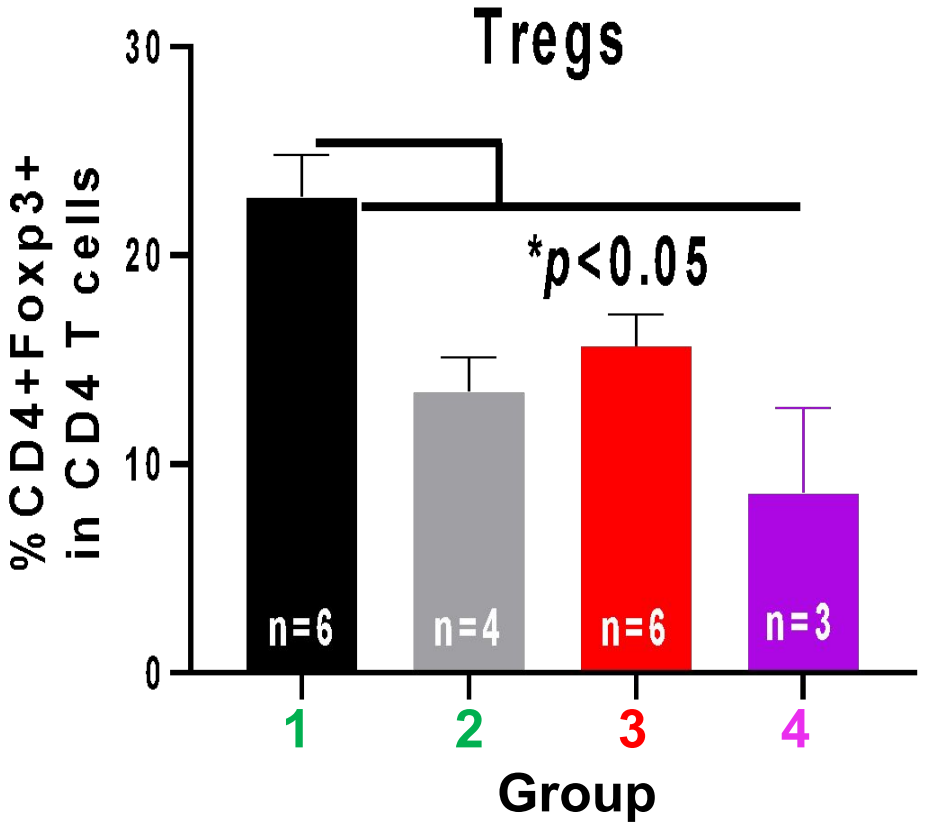


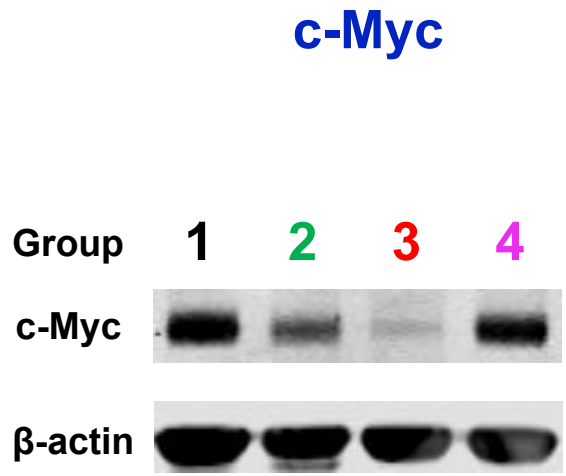
Unpublished

Treg levels and c-Myc expression in tumor after treatment vs. PBS

Group Treatment

1. P/AP-2/CD-20 Fab'
2. P/AP-2/H6/c-Myc AON
3. P/AP-2/H6/c-Myc AON+αPD-1
4. P/AP-2/CD20 Fab'+αPD-1

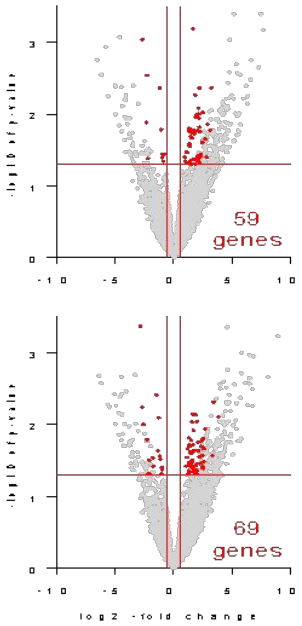


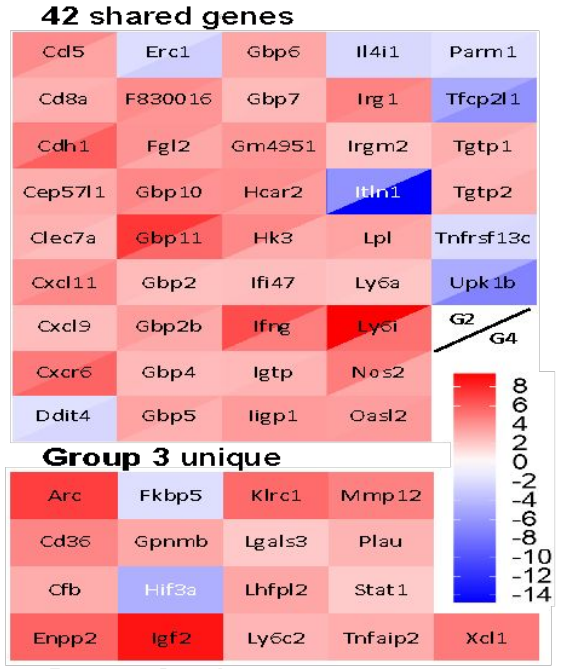


AI analysis demonstrates M1-MΦ associated gene expression

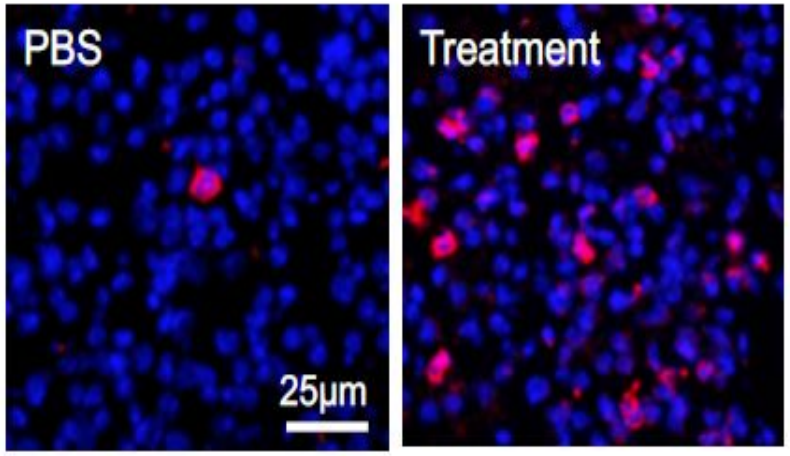
Group	Treatment
1	PBS
2	P/AP-2/CD-20 Fab’
3	P/AP-2/H6/cMyc AON
4	P/AP-2/H6/cMyc AON+αPD-1
5	P/AP-2/CD20 Fab’+αPD-1

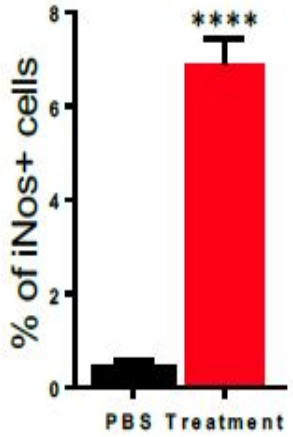
A. Heatmap Gene expression

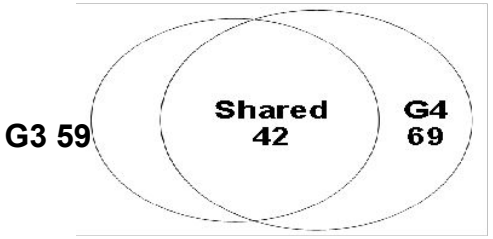




B. MΦ1 macrophage marker iNOS/NOS2 expression in tumors

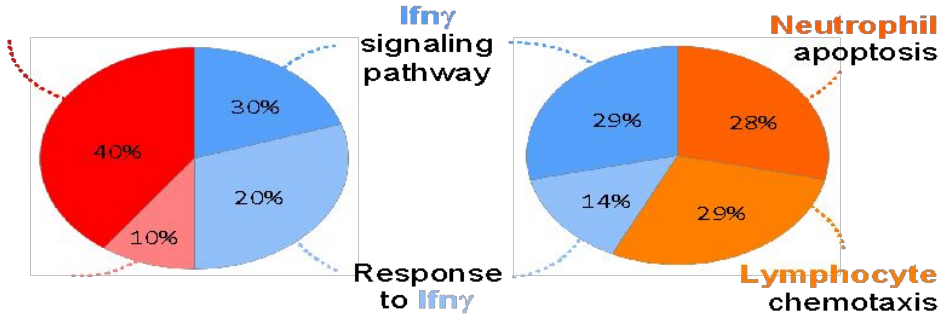




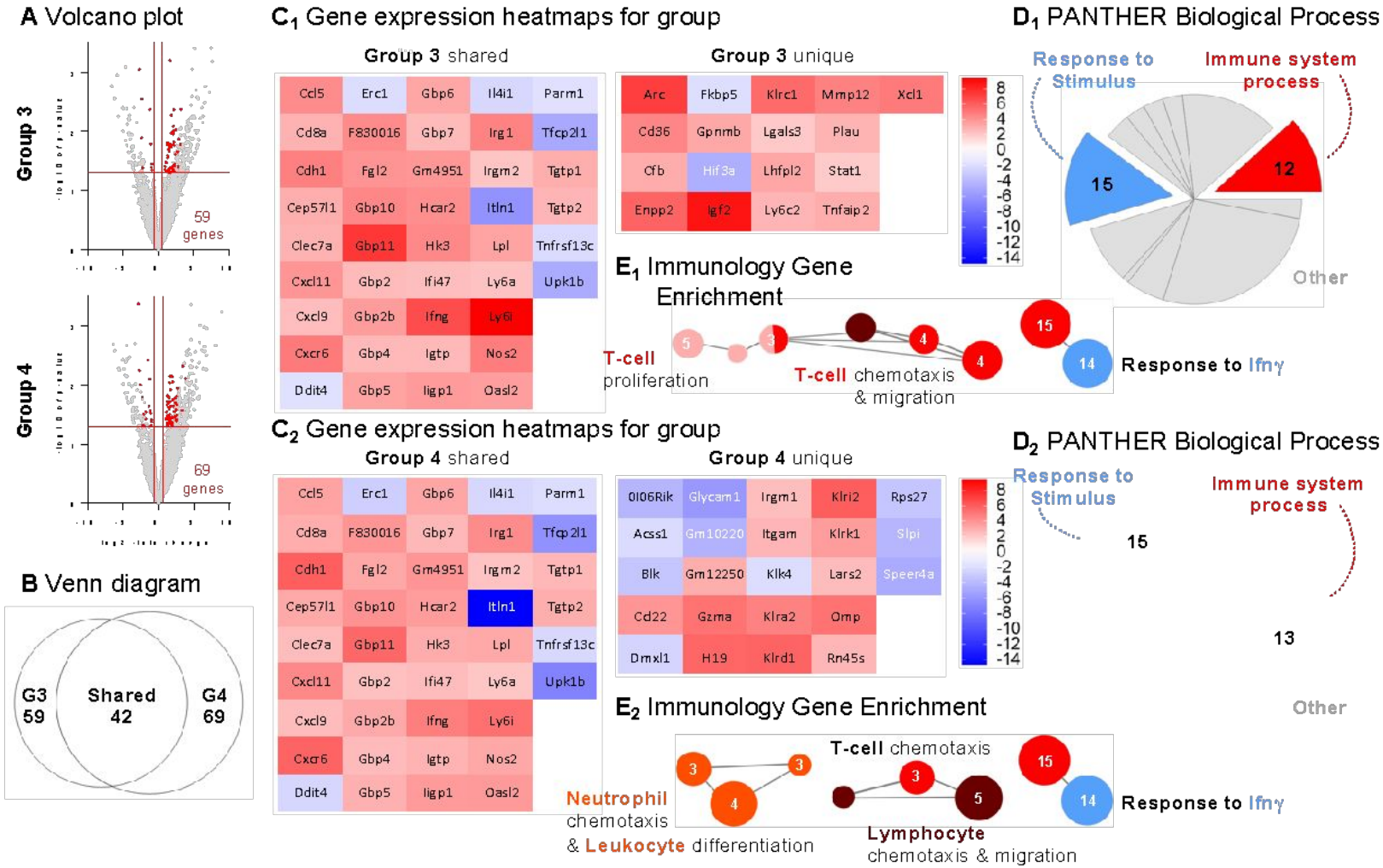


F. M1-MΦ associated genes in both Group 3 and Group 4 differentially expressed genes (DEGs)

Gene ID	CCL5	CD36	CXCL9	CXCL11	IFNγ	iNOS	Stat1
M1-MΦ associated	Yes	Yes	Yes	Yes	Yes	Yes	Yes

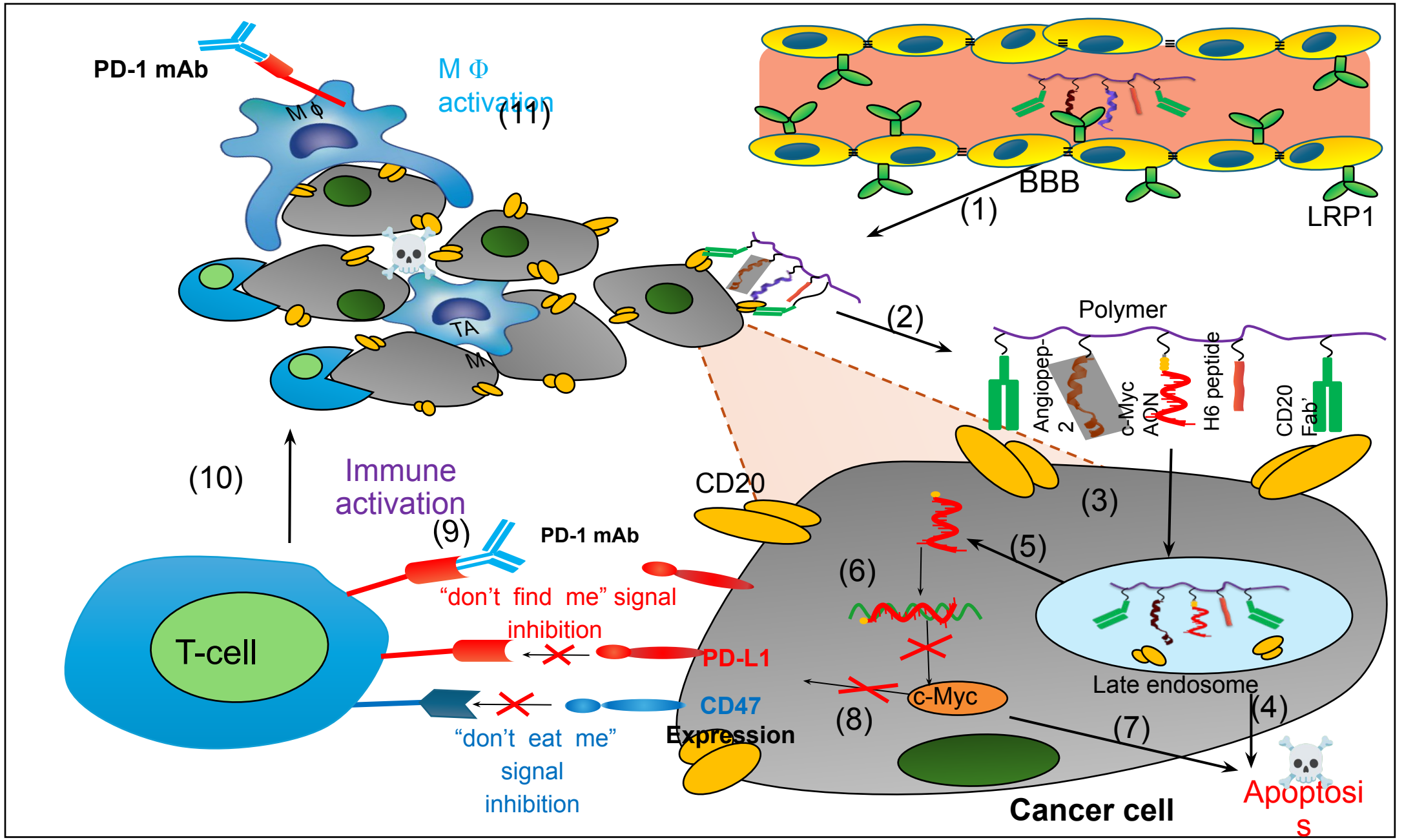


AI analyses of lymphoma transcriptome in treatment response to groups G3 and G4



A. Volcano plots show total tumor transcriptomes of 24055 detected genes with **B.** 59 and 69 differentially expressed genes in each treatment. **C. Heatmaps** of differentially expressed genes in G3 and G4, and **Venn diagram** of gene expression showing overlap between G3 and G4 treatments. **D.** PANTHER (protein analysis) of gene/protein families and their functionally related subfamilies. **E.** E1 and E2 Immunology gene enrichment analyses show equal response to Ifnγ.

Nano NIC for treatment of primary central nervous system lymphoma (summary)



Conclusions

1. *New nano immunoconjugates (NICs)* are designed to overcome biological barriers for efficient delivery of immune stimulators and anti-cancer agents to the tumor site/cells with BBB-penetrating peptides and tumor-targeted Fab's.

2. *NICs* have *new chemical features,* contain multiple AP-2 peptides for efficient delivery across the BBB and

   a) Fab' fragments of αCD20 human Ab for crosslinking of CD20 receptors on PCNSL tumor cells resulting in apoptosis;

   b) C-Myc inhibitor bound via a disulfide bond is transferred to the cytoplasm followed by S-S cleavage; this is facilitated by $(His)_6$ peptide-mediated disruption of endosomes.

3. *New combination strategy for PCNSL treatment* was designed based on compatibility of mechanisms and multipronged action: combination of (a) c-Myc inhibition, (b) CD20 crosslinking and (c) PD-1 receptor blocking.

4. *We explored* the efficacy of BBB-crossing *NICs* combined with cytotoxic T cell activating αPD-1 Ab to additionally boost both systemic and *local anti-tumor immune response*.

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