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INTRODUCTION & AIM

STUPP THERAPY: Median survival 12-15 months (2).



Surgery

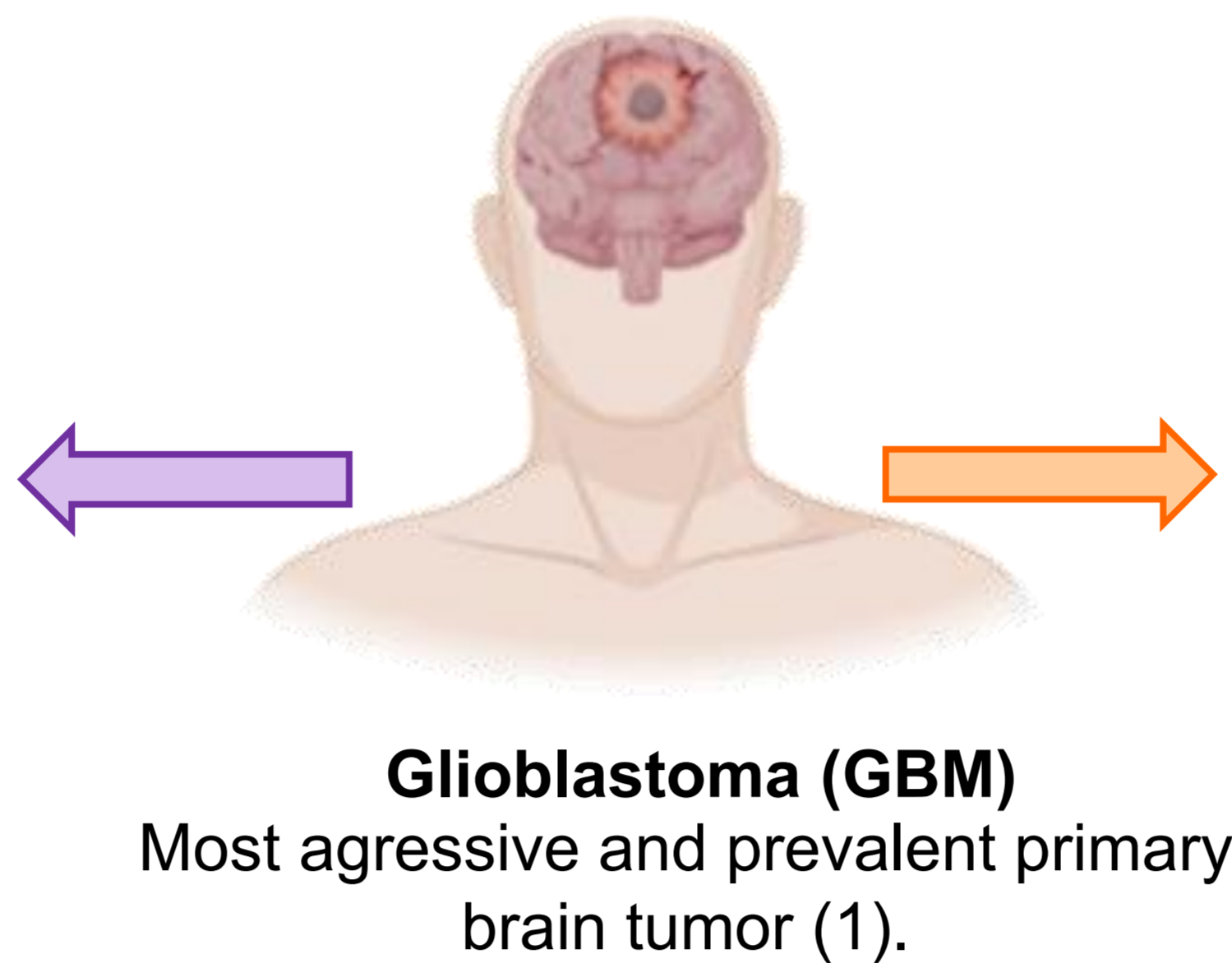


Radiotherapy

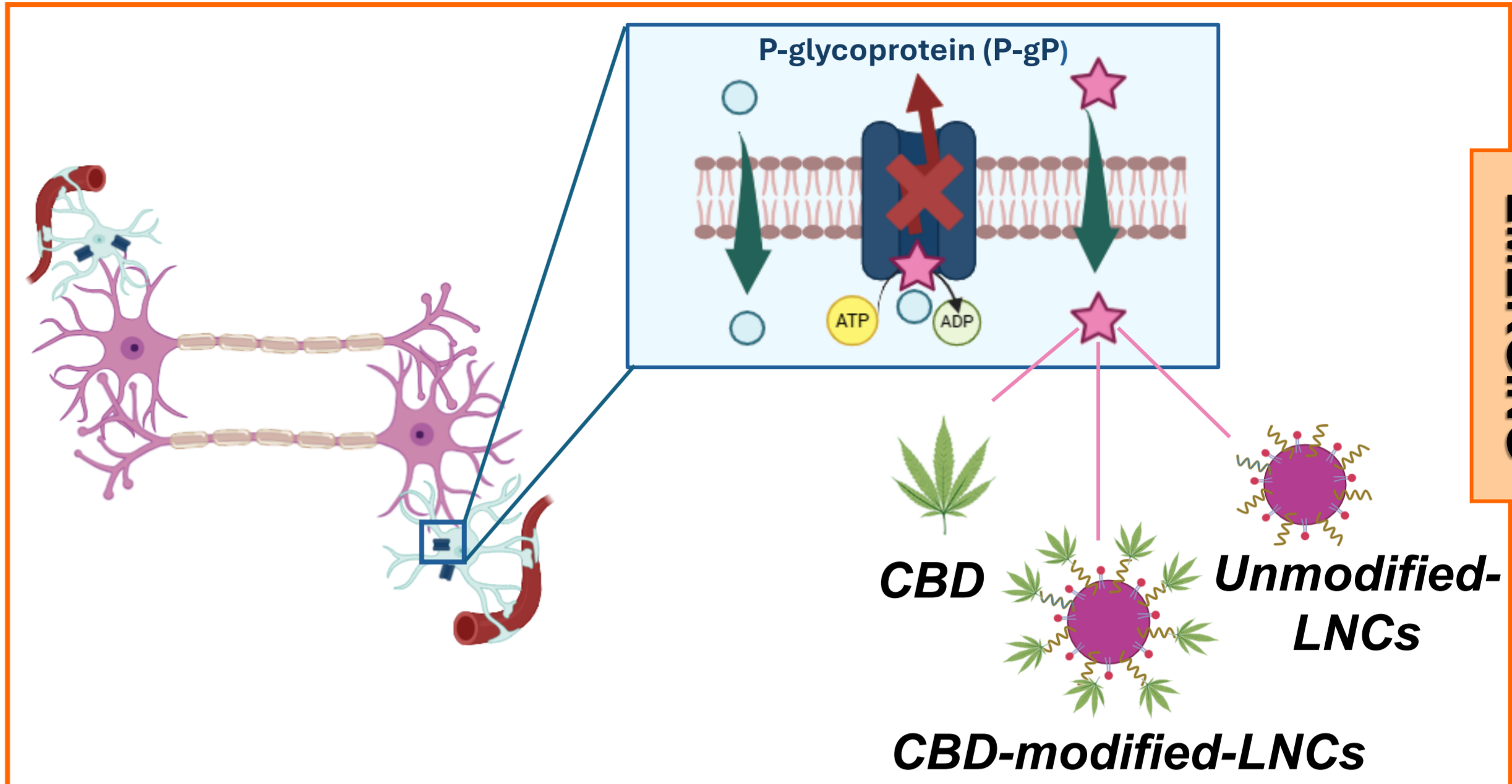


Chemotherapy
(temozolomide)

TREATMENT CHALLENGES



Glioblastoma (GBM)
Most aggressive and prevalent primary brain tumor (1).



EMERGING

METHODOLOGY

1) Lipid Nanocapsules (LNCs) formulation

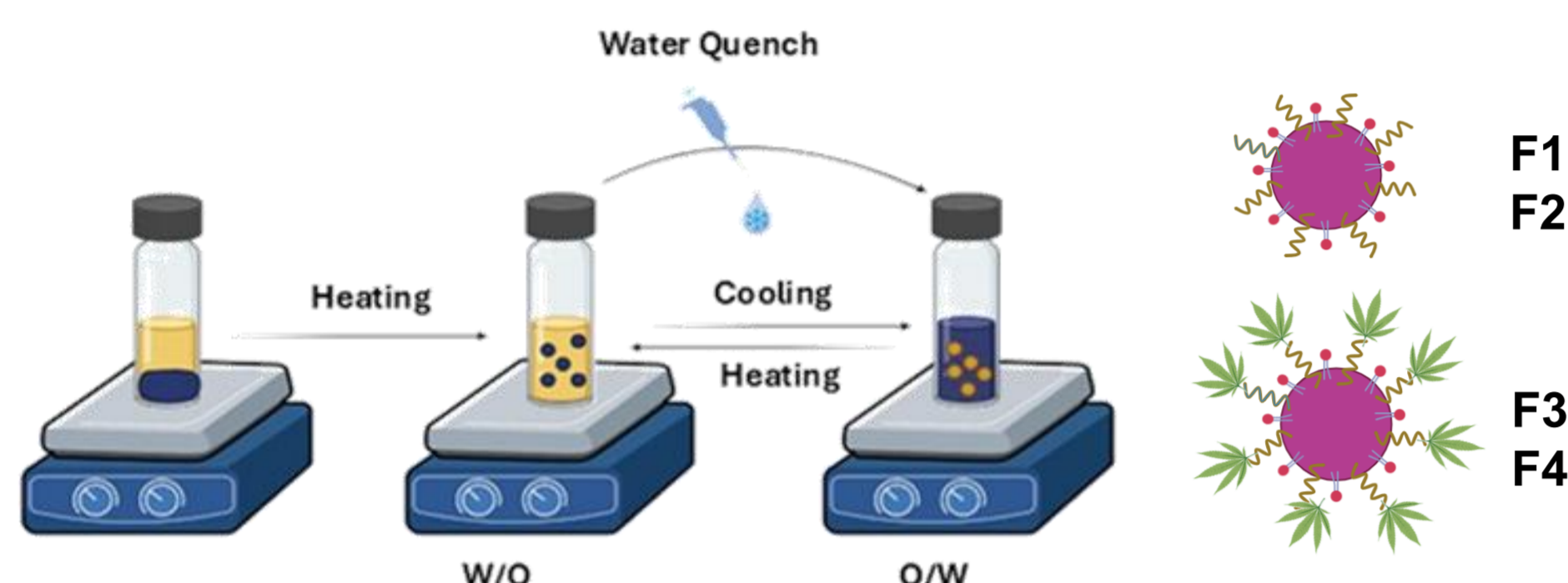


Figure 1. LNCs preparation by phase inversion temperature method (3).

[CBD] (μM)	[F1] (μg/ml)	[F2] (μg/ml)	[F3] (μM)	[F4] (μM)
2.5	86.7	129	2.8	2.8
5	173	256	5.6	5.6
10	346	513	11.2	11.2
15	520	770	16.8	16.8

Table 1. Equivalences between the tested concentrations of free CBD, Unmodified-LNCs (F1 and F2), and CBD-functionalized LNCs (F3 and F4).

2) P-gp activity

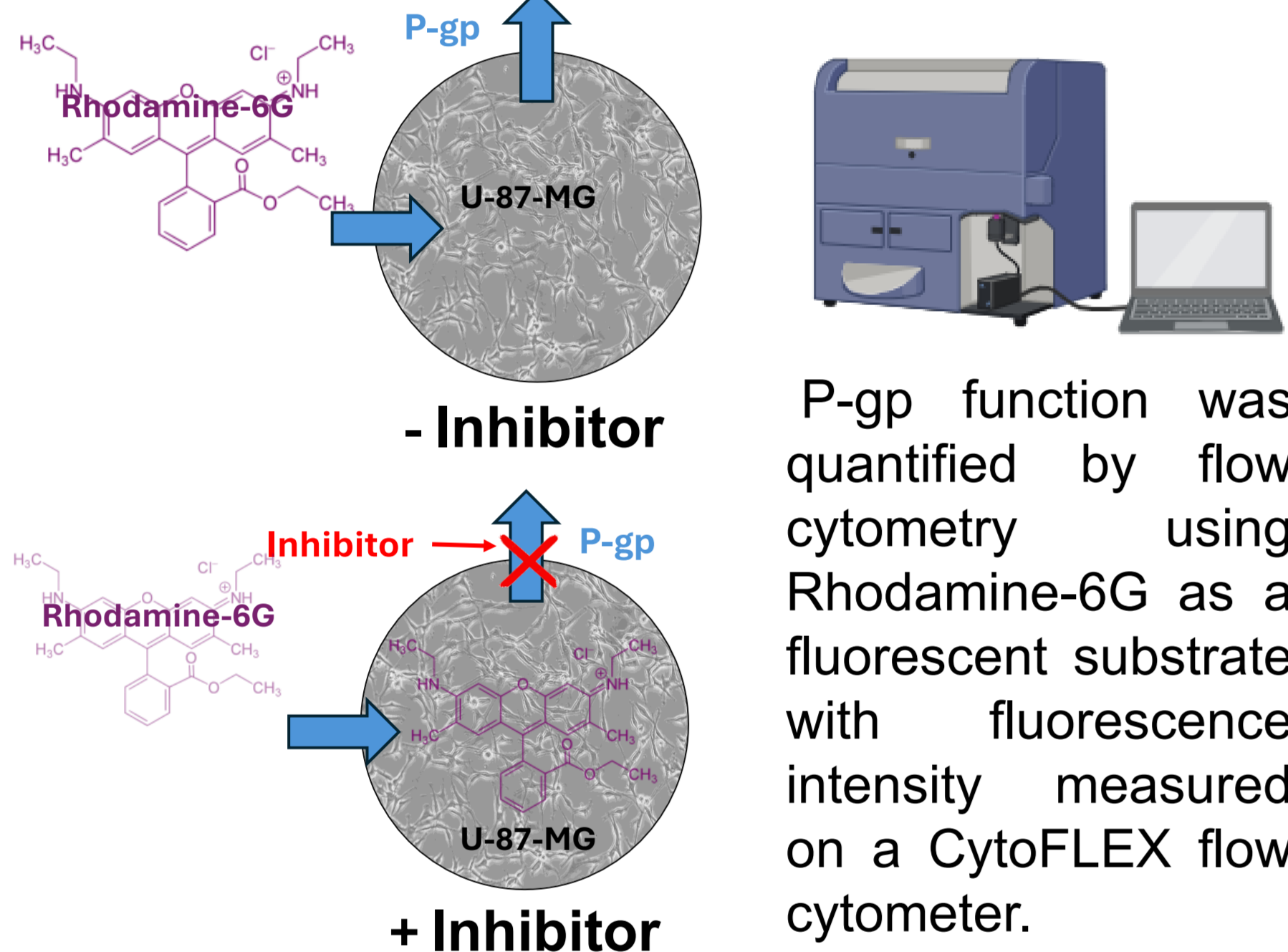
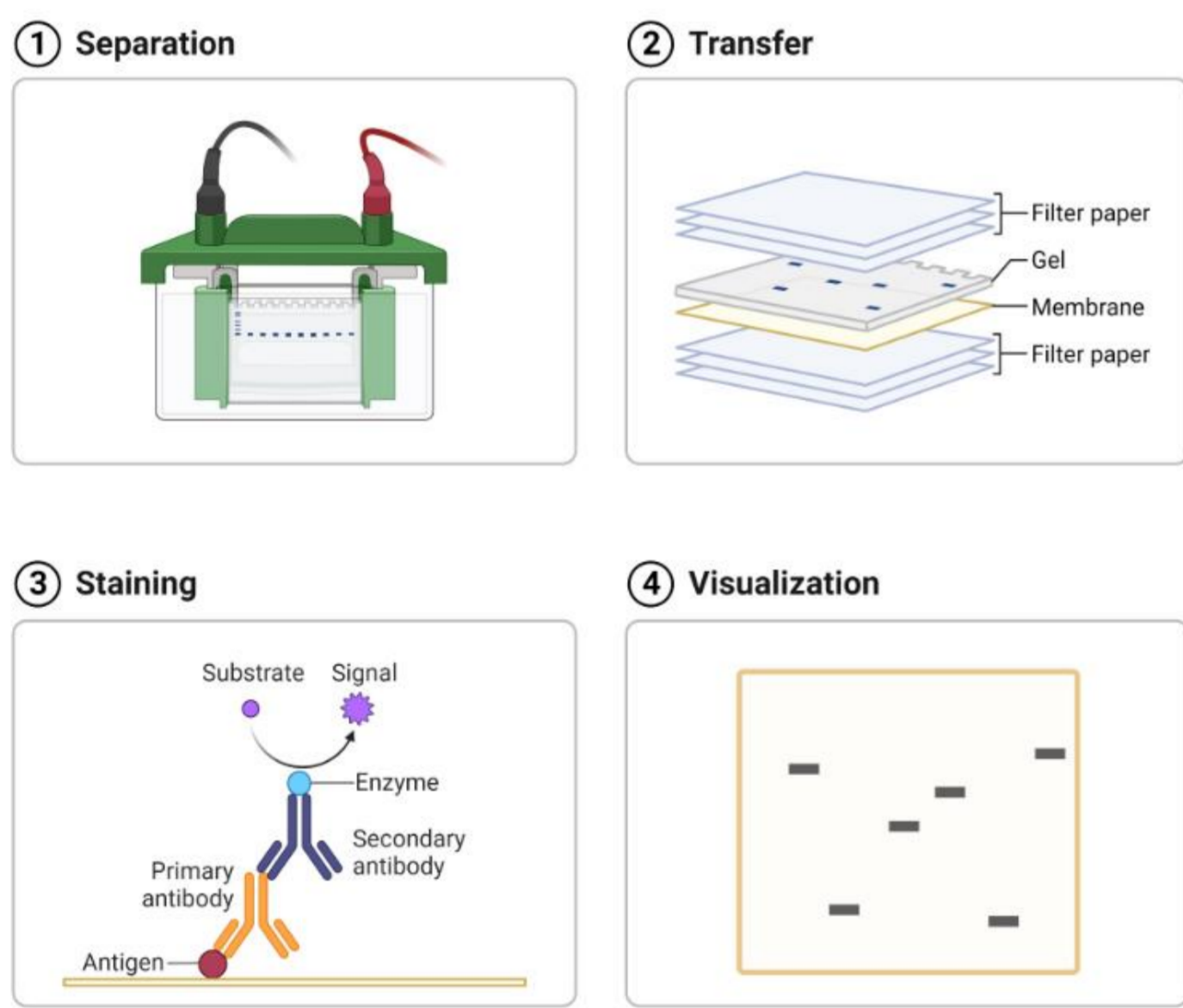


Figure 2. P-gp function assessed by Flow Cytometry

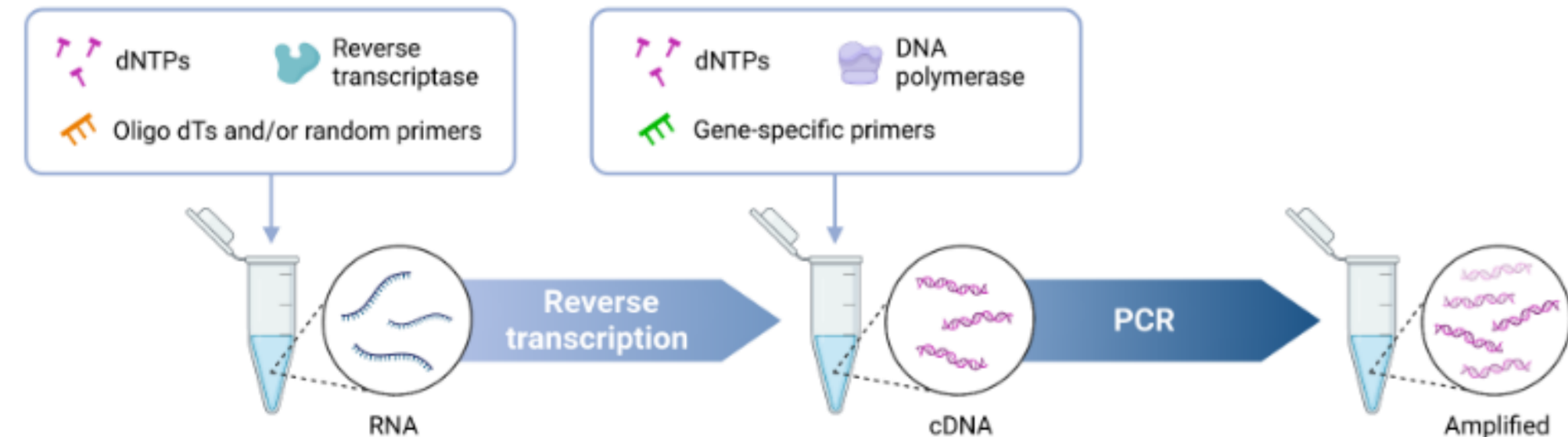
3) P-gp Protein expression



Protein expression levels were assessed by Western blot analysis, using chemiluminescent detection with an ImageQuant LAS 500 CCD camera, and semi-quantitative analysis was performed with ImageJ software.

Figure 3. P-gp protein expression assessed by Western Blot.

4) P-gp gene expression



ABCB1 gene expression was evaluated by RT-qPCR analysis. Amplifications were run in a 7900 HT-Fast Real-Time PCR System. Relative gene expression expression was calculated using the $2^{-\Delta\Delta C_t}$ method.

Figure 4. P-gp gene expression assessed by RT-qPCR.

EXPERIMENTAL RESULTS

1) LNCs characterization

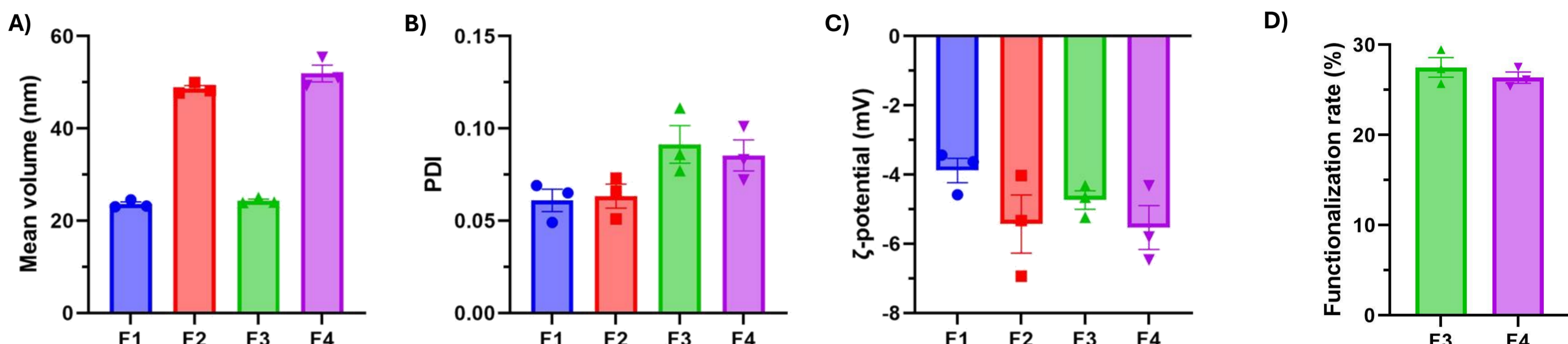
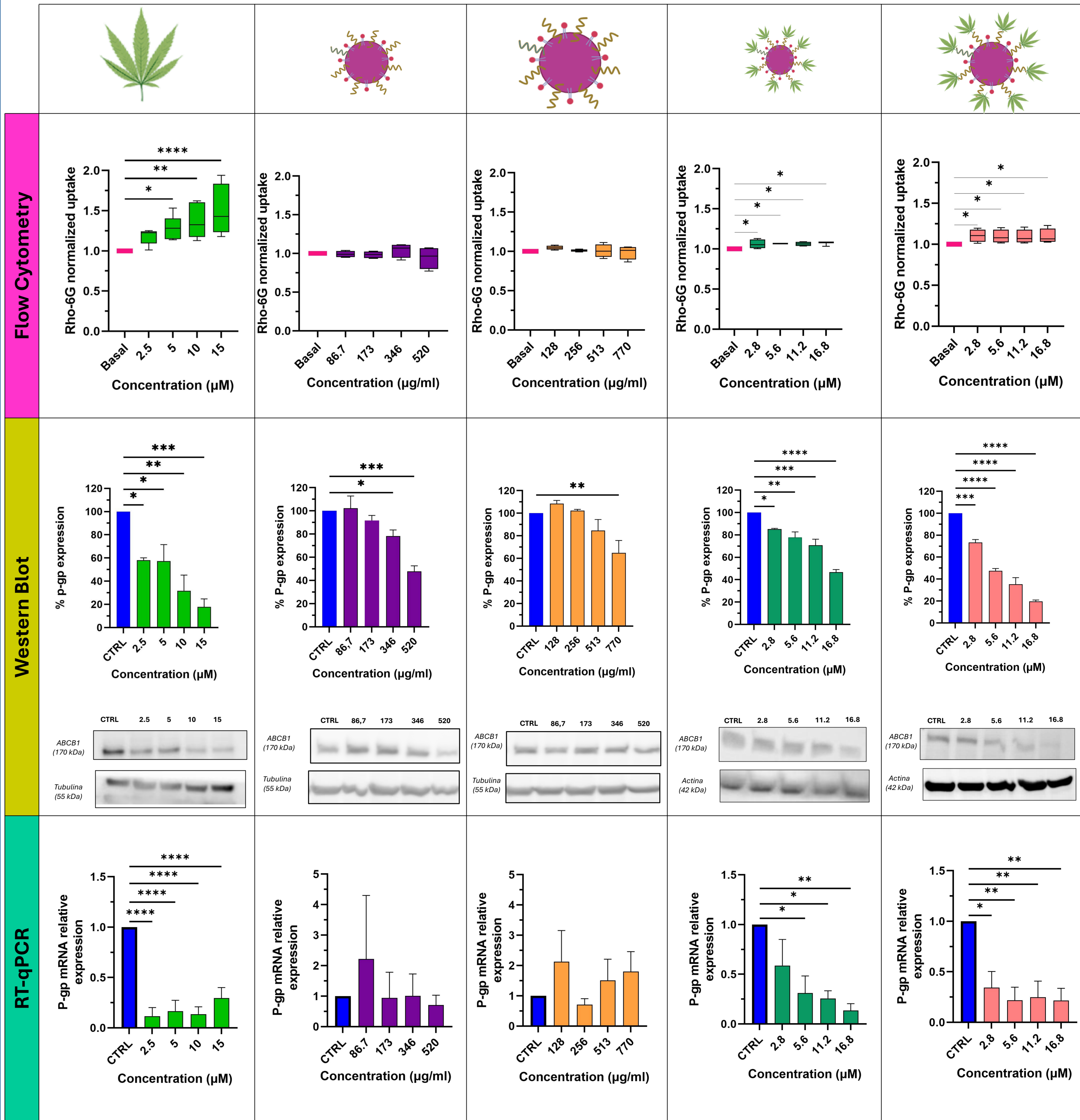
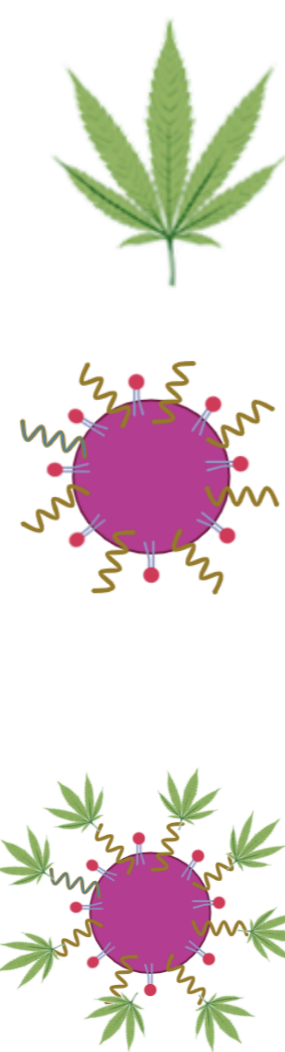


Figure 5. Size (A), polydispersity index (B), ζ -potential (C) analysis of the different LNC formulations (N=3). Percentage (%) of functionalization rate in CBD-modified LNCs (D).

2) Targeting P-gp: Comparative effects of CBD, Unmodified-LNCs, and CBD-modified-LNCs



CONCLUSIONS



CBD effectively inhibited P-gp function and reduced its protein and gene expression in a concentration-dependent manner, supporting its potential as an adjuvant to improve drug delivery in GBM chemotherapy.

Unmodified-LNCs did not affect P-gp function or gene expression but induced a downregulation of P-gp protein expression, likely through membrane interaction.

CBD-modified LNCs integrate the dual benefits of CBD and advantageous delivery profile of LNCs, achieving coordinated inhibition of P-gp at the three studied levels. The enhanced effect observed with the larger formulation suggests improved CBD surface presentation. These nanocarriers offer a promising and innovative strategy to overcome multidrug resistance in GBM, positioning them as a powerful tool for more effective CNS chemotherapy.

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BIBLIOGRAPHY



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