Therapeutic potential of EC-18 as a chemotherapy adjuvant for 5-fluorouracil-induced neutropenia

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Abstract

Chemotherapy-induced neutropenia (CIN) is a complication that arises during cancer treatment and necessitates dose reduction. Preventing CIN and maintaining absolute neutrophil counts (ANC) is critical for successful chemotherapy because a rapid decline of neutrophils increases susceptibility to infection. Here, we investigated whether administration of EC-18 has therapeutic effects on the treatment of CIN in 5-fluorouracil (5-FU)-induced neutropenia mouse model. A single injection of 5-FU 100mg/kg reduced the ANC in the control, EC-18 125 and EC-18 250mg/kg-treated cohort from pre-injection values to <500 cells/μL by 5.2±0.45, 5.8±0.45 and 5.8±0.45 days, respectively. The administration of EC-18 in 5-FU-injected mice resulted in significant reduction in the duration of neutropenia and the time to recovery of ANC >1000 cells/μL. EC-18 125 or 250mg/kg significantly reduced the duration of neutropenia from 7.4±1.14 days to 2.6±0.55, 3.0±0.71 days, respectively. Moreover, the ANC of all individuals in the control cohort fell to severely neutropenic range (ANC <100 cells/ μL), while only 20% of individuals in both EC-18 125 and 250mg/kg-treated cohorts experienced severe neutropenia. EC-18 also reduced the duration of severe neutropenia from 5.2±1.48 days to 2 days. EC-18 125 or 250mg/kg administration significantly increased the mean nadir after 5-FU injection from 2.0±4.47 cells/μL to 236±4.47 or 158±11.32 cells/μL, respectively. The time of recovery to an ANC > 500 or 1000 cells/μL was significantly reduced in EC-18 125 and 250mg/kg-treated cohorts. Besides neutropenia, a single treatment of 5-FU induced the reduction of blood monocytes and eosinophils, similar to the pattern of the decrease of neutrophil counts. The administration of EC-18 125 or 250mg/kg in 5-FU-injected mice remarkably prevented the reduction of blood monocytes and eosinophils. In this study, thrombocytopenia is defined as a 50% or greater reduction in platelet count from baseline, and 2-fold or greater increase of platelet count from baseline for thrombocytosis. 5-FU treatment induced the moderate thrombocytopenia from 4 to 6 days and followed by a more pronounced and prolonged rebound thrombocytosis. EC-18 significantly reduced the extreme change in platelet counts, thus preventing 5-FU-induced thrombocytopenia and thrombocytosis. Moreover, EC-18 effectively prevented a constant reduction of red blood cell (RBC) count induced by 5-FU treatment. Based on the observations in this study, we concluded that EC-18 has therapeutic potential as a chemotherapy adjuvant for the treatment of 5-FU-induced CIN as well as chemotherapy-associated other hematologic disorders.

Introduction

- Chemotherapy-induced neutropenia (CIN) is a complication that arises during cancer treatment and necessitates dose reduction. **Caggiano V, Weiss RV, Rickert TS, Linde-Zwirble WT. Cancer 2005;103:1916-24.**

- Preventing CIN and maintaining absolute neutrophil counts is critical for successful chemotherapy because a rapid decline of neutrophils increases susceptibility to infection. **Santolaya ME, Alvarez AM, Becker A, Cofre J, Enriquez N, O'Ryan M, et al. J Clin Oncol 2001;19:3415-21**

- In previous study, EC-18 attenuated gemcitabine-induced neutropenia via regulation of neutrophil extravasation. **Jeong et al. Cell Biosci. 2019; 9: 4. (Published online 2019 Jan 3. doi: 10.1186/s13578-018-0266-7).**
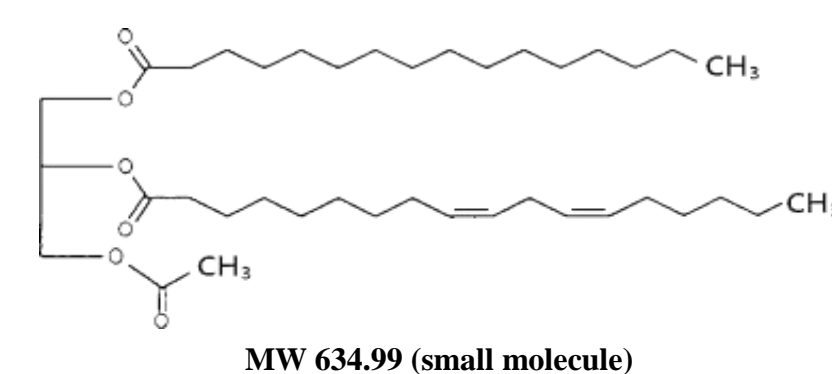
EC-18 (PLAG: 1-Palmitoyl-2-Linoleoyl-3-Acetyl-rac-Glycerol)
MW 634.99 (small molecule)

Results

1. Therapeutic effect of administration of EC-18 on the treatment of neutropenia in 5-FU-induced neutropenia mouse model.

Harvest total blood and CBC analysis
EC-18
5-FU
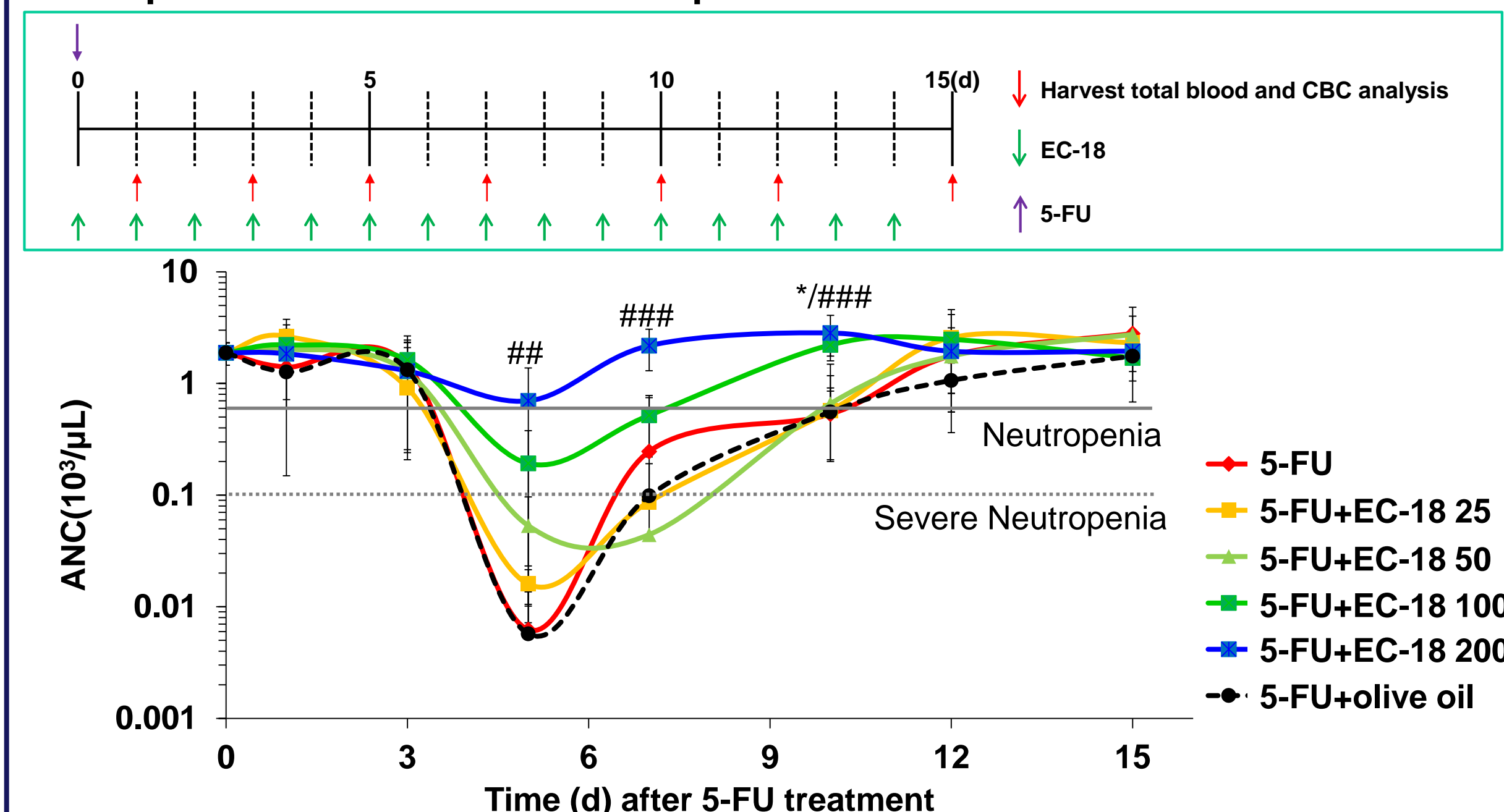
Effect of administration of EC-18 on the mean absolute neutrophil counts of mice following injection with 100mg/kg of 5-fluorouracil. * 5-FU vs. 5-FU+EC-18 100 mg/kg, # 5-FU vs. 5-FU+EC-18 200 mg/kg; */#l

5-FU
5-FU+EC-18 25
5-FU+EC-18 50
5-FU+EC-18 100
5-FU+EC-18 200
5-FU+olive oil
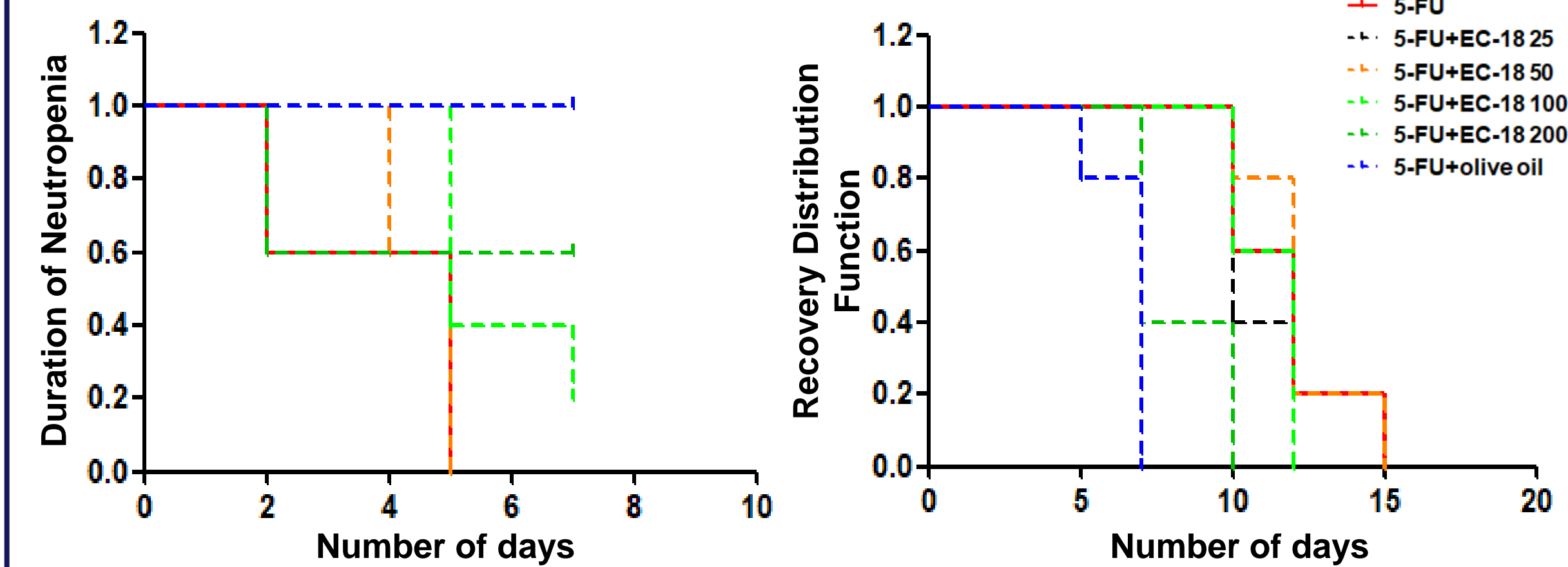
Effect of administration of EC-18 on the duration of neutropenia and on time to recovery to ANC <500 cells/μL.

Table 1. Mean First Day of Neutropenia (ANC <500 cells/μL), Mean Duration of Neutropenia, Number of Individuals of Severe Neutropenia (ANC <100 cells/μL), and Mean Duration of Severe Neutropenia in Control, and EC-18 25, 50, 100, 200 and olive oil-treated mice injected with 5-FU 100mg/kg

Treatment	Mean First Day Neutropenia (± SE, range)	Mean Duration of Neutropenia in Days (± SE, range)	Number of Individuals of Severe Neutropenia	Mean Duration of Severe Neutropenia in Days (± SE, range)
Control	4.6±0.4 (3-5)	6.6±0.75 (5-9)	5/5	3.8±0.7 (2-5)
EC-18 25mg/kg	3.8±0.49 (3-5) (P = NS)	8.4±1.17 (5-12) (P = NS)	5/5	4.6±0.2 (4-5)
EC-18 50mg/kg	5.0±0.0 (5-5) (P = NS)	6.2±0.60 (5-7) (P = NS)	4/5	5.5±0.5 (5-7)
EC-18 100mg/kg	5.4±0.4 (5-7) (P = NS)	2.6±0.60 (2-5) (P = 0.0031)	2/5	2.0±0.0 (2-2)
EC-18 200mg/kg	4.6±0.4 (3-5) (P = NS)	2.0±0.0 (2-2) (P = 0.001776)	0/5	N/A
Olive oil	5.0±0.0 (5-5) (P = NS)	5.8±0.49 (5-7) (P = NS)	5/5	3.8±0.7 (2-5)

Treatment	Nadir of ANC (cells/μL)	Mean Number of Days to Recovery – ANC ≥500/μL (± SE, range)	Mean Number of Days to Recovery – ANC ≥1000/μL (± SE, range)
Control	6±6.0	11.8±0.9 (10-15)	12.6±0.6 (12-15)
EC-18 25mg/kg	14±2.4 (P = NS)	12.2±0.8 (10-15) (P = NS)	12.2±0.8 (10-15) (P = NS)
EC-18 50mg/kg	42±22.9 (P = NS)	11.2±0.5 (10-12) (P = NS)	10.0±0.0 (10-10) (P = 0.0061)
EC-18 100mg/kg	168±75.0 (P = NS)	8.2±0.7 (7-10) (P = 0.0155)	10.0±0.0 (10-10) (P = 0.0061)
EC-18 200mg/kg	356±52.2 (P = 0.0002)	6.6±0.4 (5-7) (P = 0.0008)	7.2±0.8 (5-10) (P = 0.0006)
Olive oil	8±3.7	10.8±0.5 (10-12)	12.8±1.0 (10-15)

Table 2. Mean Nadir and Recovery from Neutropenia in Control, EC-18 25, 50, 100, 200 and olive oil-Treated mice injected with 5-FU 100mg/kg.
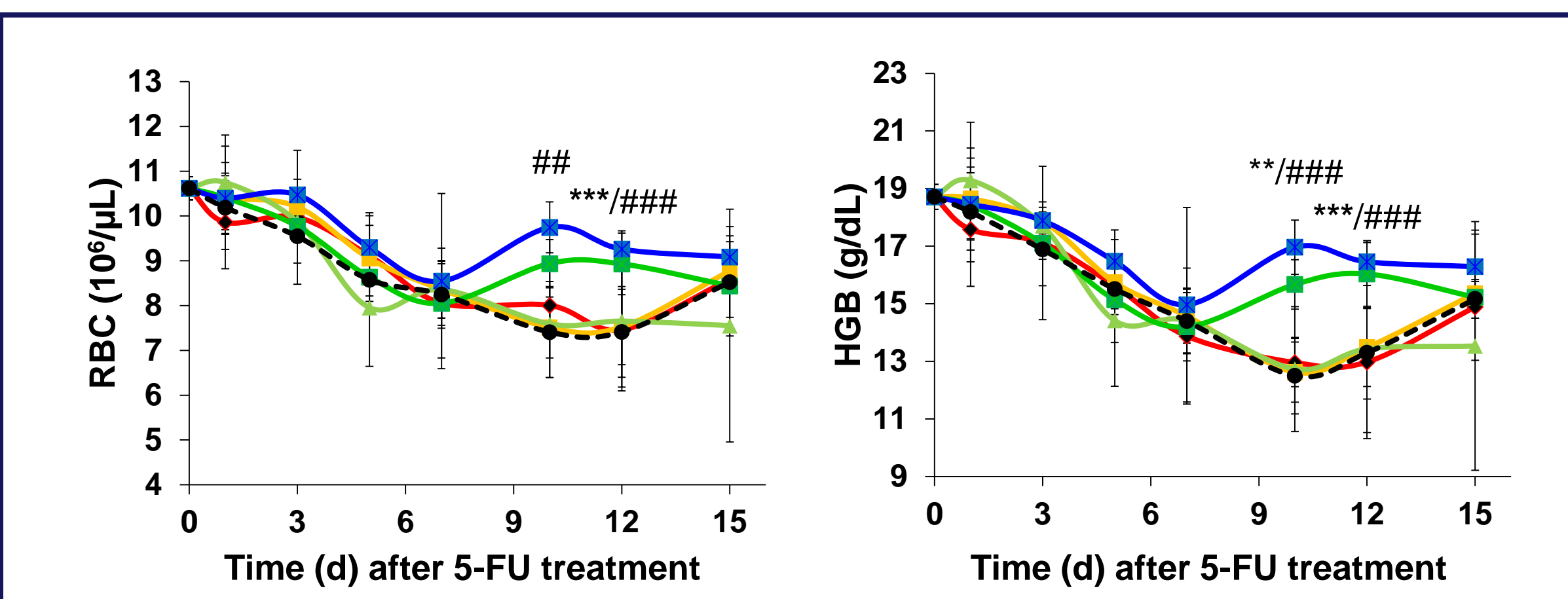
2. Therapeutic effect of administration of EC-18 on other hematologic indicators in 5-FU-treated mouse model

5-FU
5-FU+EC-18 25
5-FU+EC-18 50
5-FU+EC-18 100
5-FU+EC-18 200
5-FU+olive oil
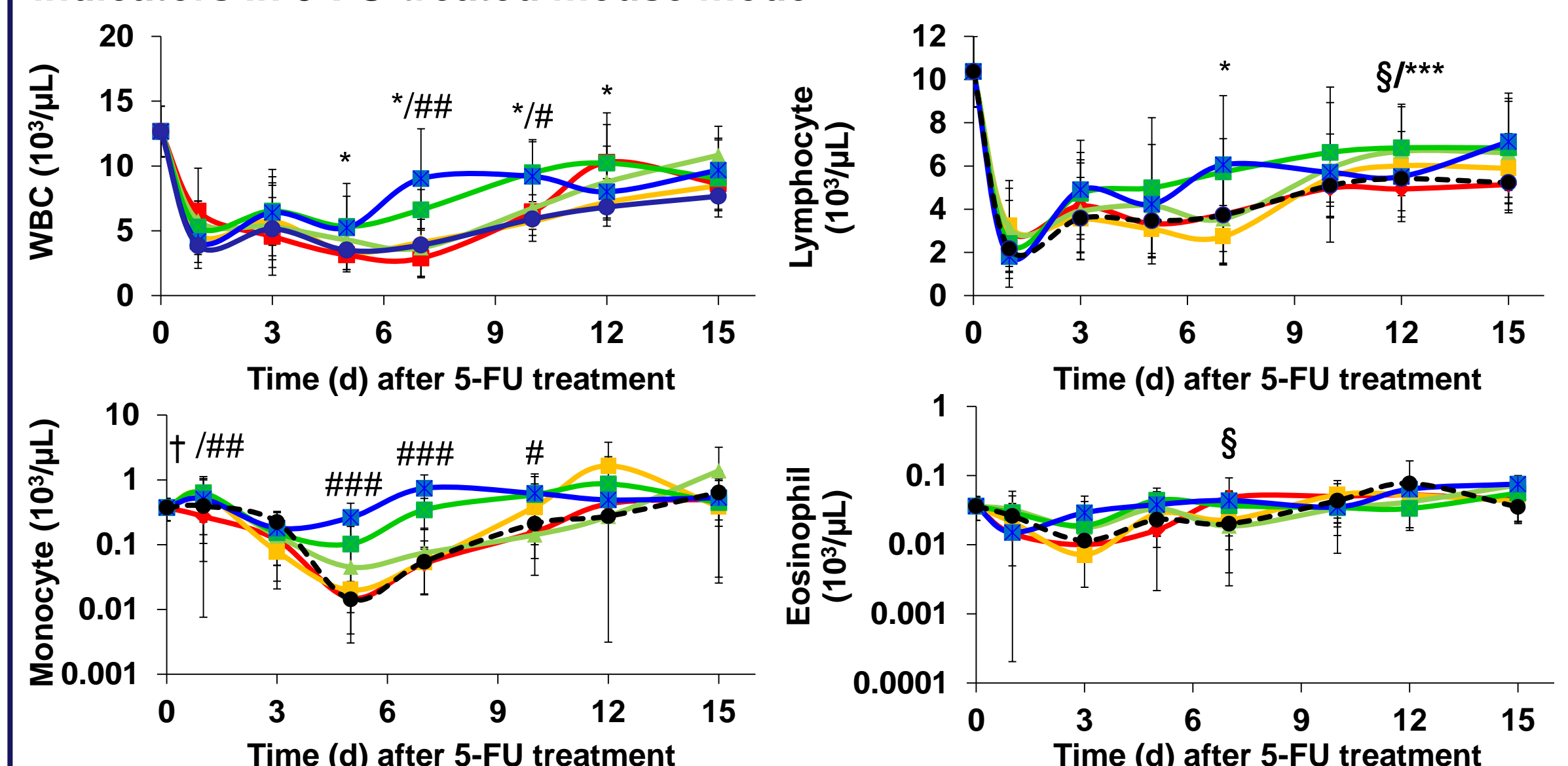
Effect of administration of EC-18 on the mean WBC, lymphocyte, monocyte and eosinophil counts of mice following injection with 100mg/kg of 5-fluorouracil. †5-FU vs. 5-FU+EC-18 25 mg/kg, §5-FU vs. 5-FU+EC-18 50 mg/kg,* 5-FU vs. 5-FU+EC-18 100 mg/kg, # 5-FU vs. 5-FU+EC-18 200 mg/kg; †/§/*/#P<0.05, ††/§§/**/##P<0.01 and †††/§§§/***/###P<0.001.
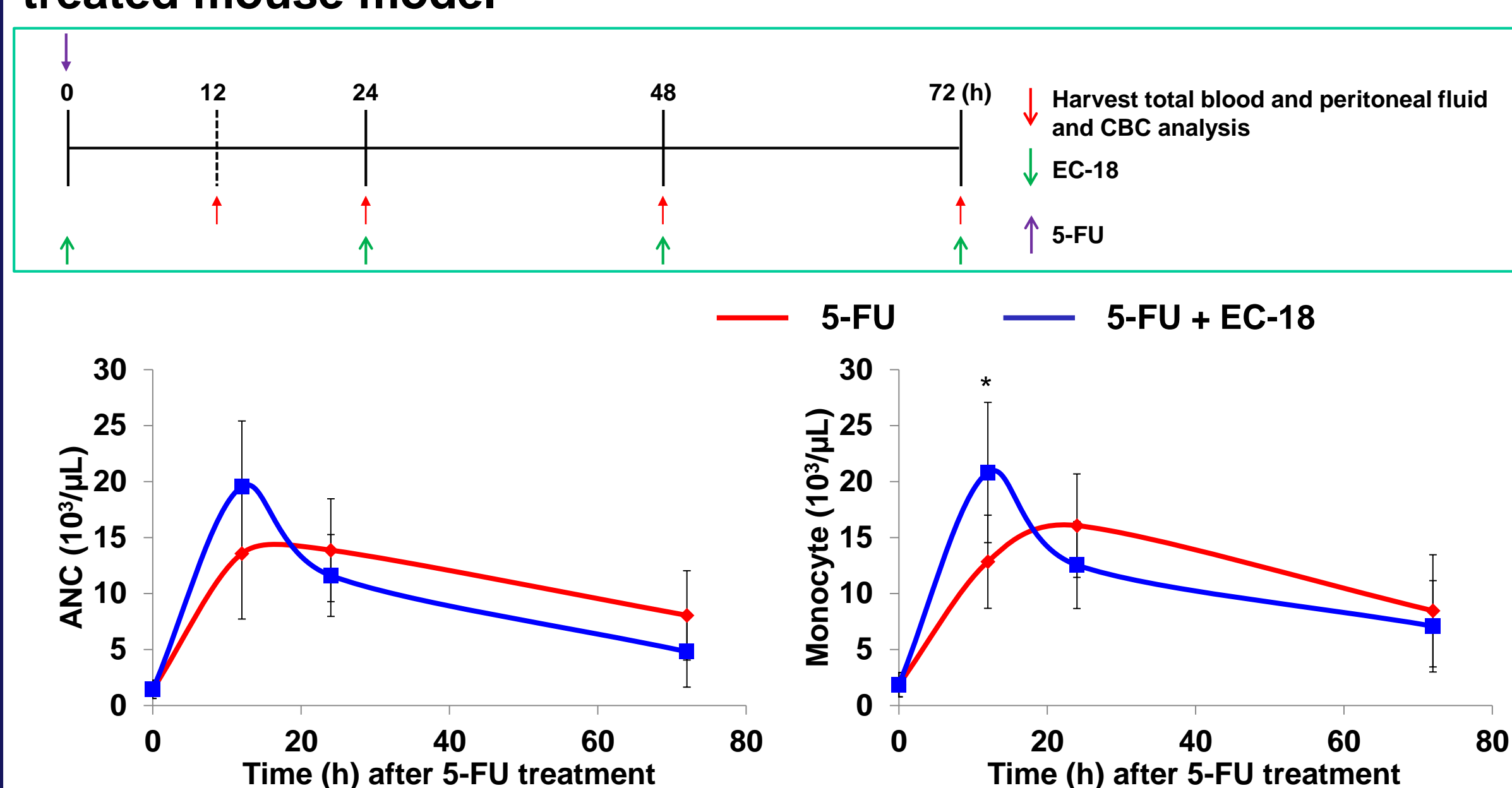

Thrombosis
50% reduction
70% reduction
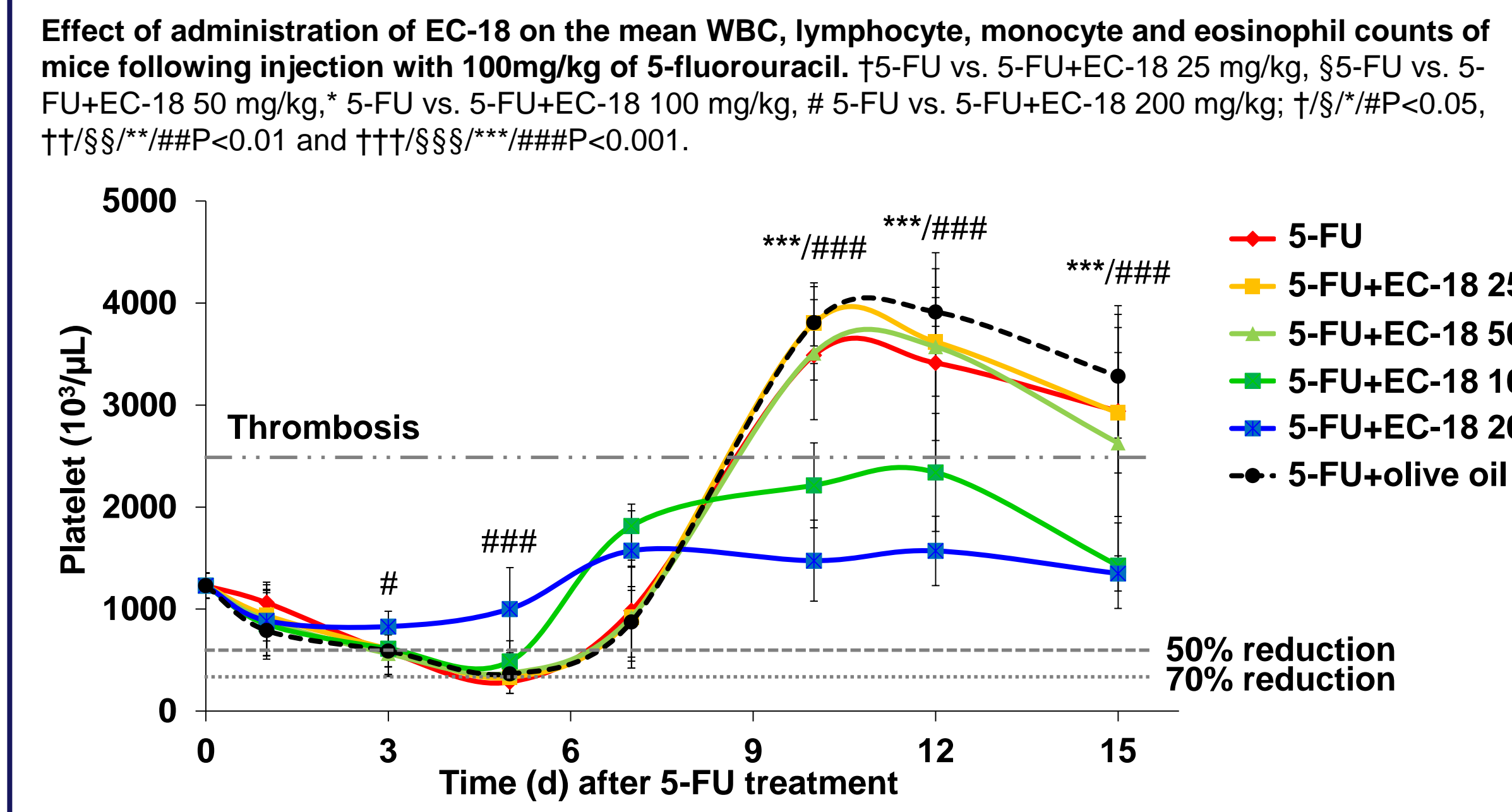
Effect of administration of EC-18 on the mean platelet counts of mice following injection with 100mg/kg of 5-fluorouracil. * 5-FU vs. 5-FU+EC-18 100 mg/kg, # 5-FU vs. 5-FU+EC-18 200 mg/kg; */#P<0.05, **/##P<0.01 and ***/###P<0.001.

Effect of administration of EC-18 on the mean RBC counts and hemoglobin levels of mice following injection with 100mg/kg of 5-fluorouracil. * 5-FU vs. 5-FU+EC-18 100 mg/kg, # 5-FU vs. 5-FU+EC-18 200 mg/kg; */#P<0.05, **/##P<0.01 and ***/###P<0.001.

3. Effect of EC-18 administration on leukocyte recruitment in 5-FU-treated mouse model

Harvest total blood and peritoneal fluid and CBC analysis
EC-18
5-FU

5-FU
5-FU + EC-18

Effect of administration of EC-18 on the mean count of neutrophils and monocytes in the peritoneal cavity following injection with 100mg/kg of 5-fluorouracil. * 5-FU vs. 5-FU+EC-18; *P<0.05

Conclusion

- Under 5-FU-induced neutropenic condition, EC-18 significantly increased the ANC and reduced the duration of neutropenia and time of recovery.

- EC-18 also effectively prevented other hematologic disorders induced by 5-FU treatment, such as the reduction of blood monocytes and eosinophils, thrombocytopenia, thrombocytosis and anemia.

- Based on the observations in this study, we concluded that therapeutic administration of EC-18 could be developed as a chemotherapeutic adjuvant for the treatment of CIN as well as chemotherapy-associated other hematologic disorders.

5-FU → Tissue damage → Damaged tissue → Recruitment of Neutrophil and Monocyte → Resolution → Hematological disease: Neutropenia Thrombocytopenia Anemia
EC-18 → Acceleration