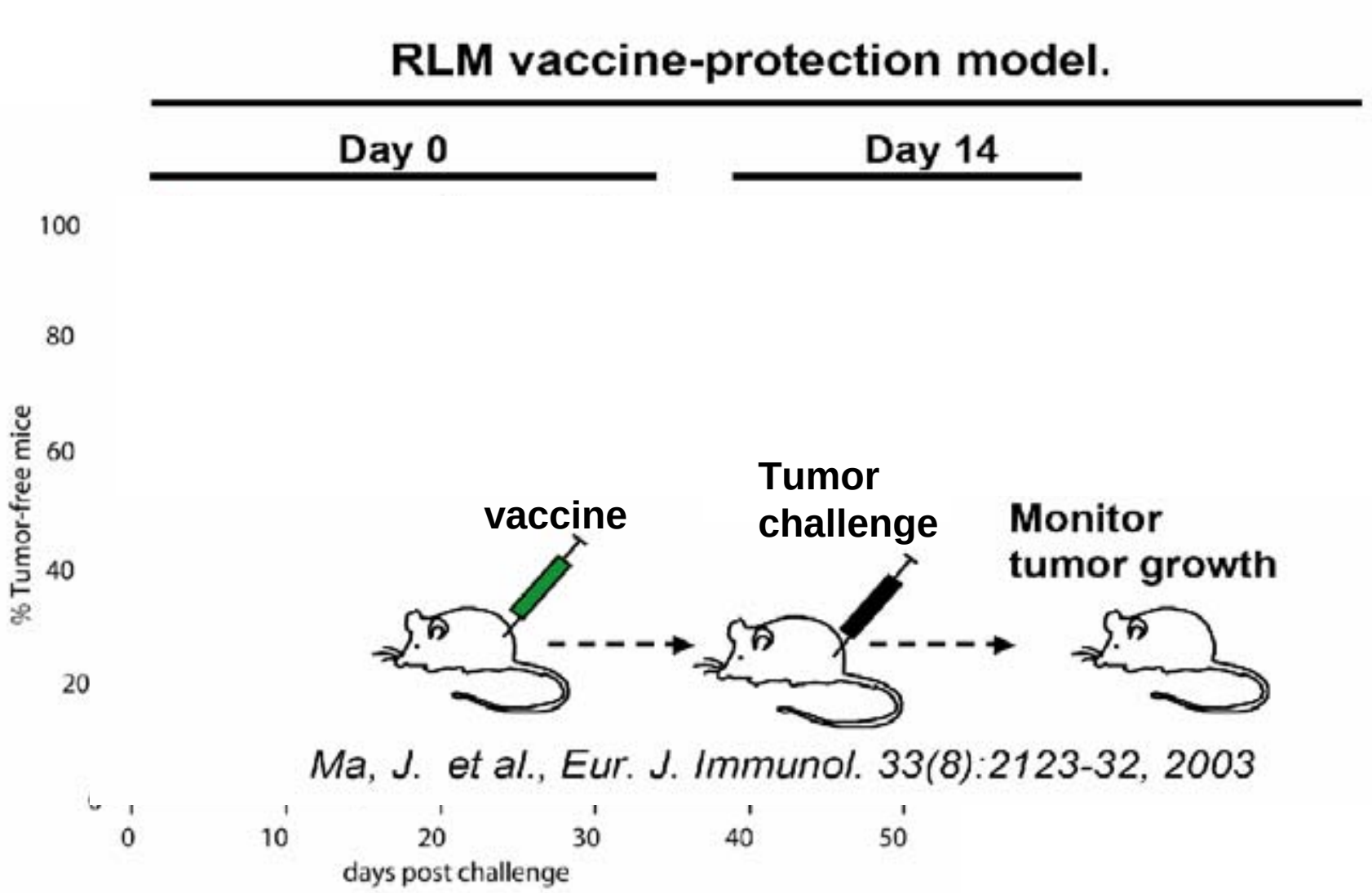
CYCLOPHOSPHAMIDE AND FLUDARABINE TREATMENT PRIOR TO RECONSTITUTION WITH PBMC DOES NOT REDUCE THE FREQUENCY OF CIRCULATING FOXP3+ CELLS IN VACCINATED PROSTATE CANCER PATIENTS

James A. Thompson

Earle A. Chiles Research Institute

Why reconstitute lymphopenic cancer patients and then vaccinate ?

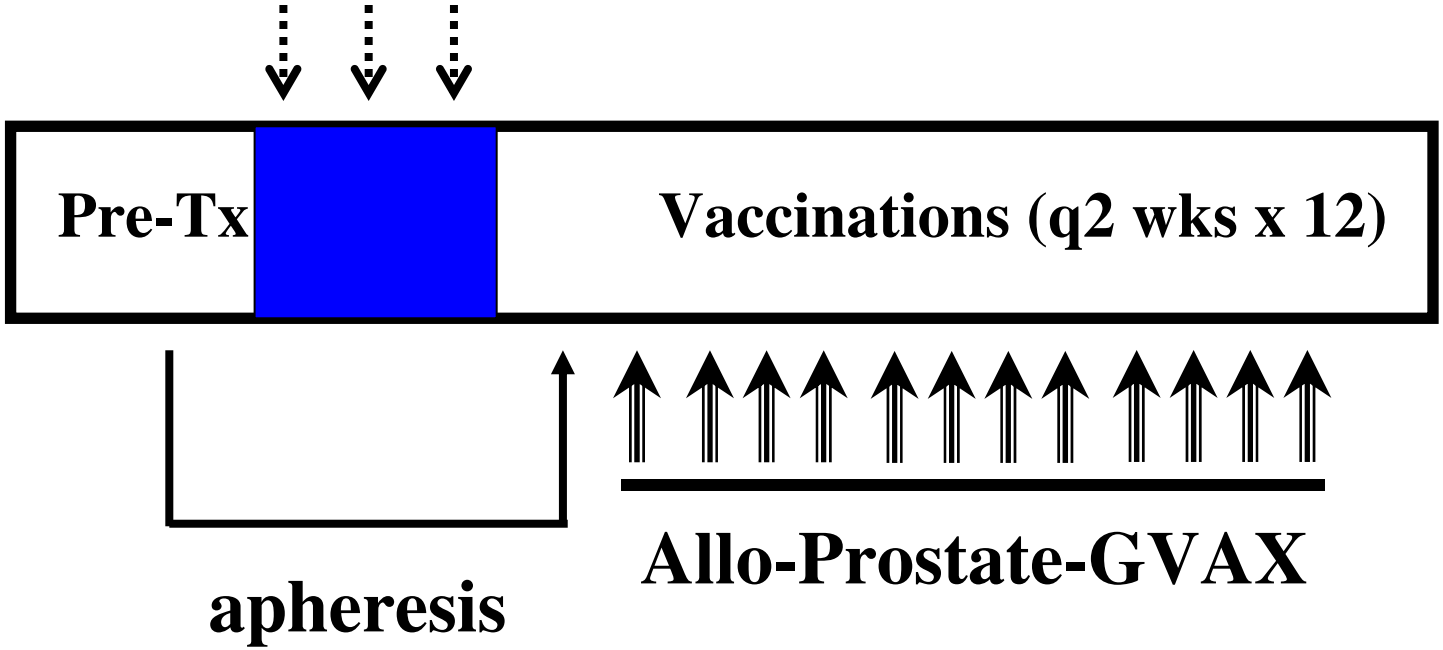


Hypothesis:

Combining lymphodepleting chemotherapy with reconstitution and vaccination will result in fewer Tregs and stronger anti-tumor immune responses.

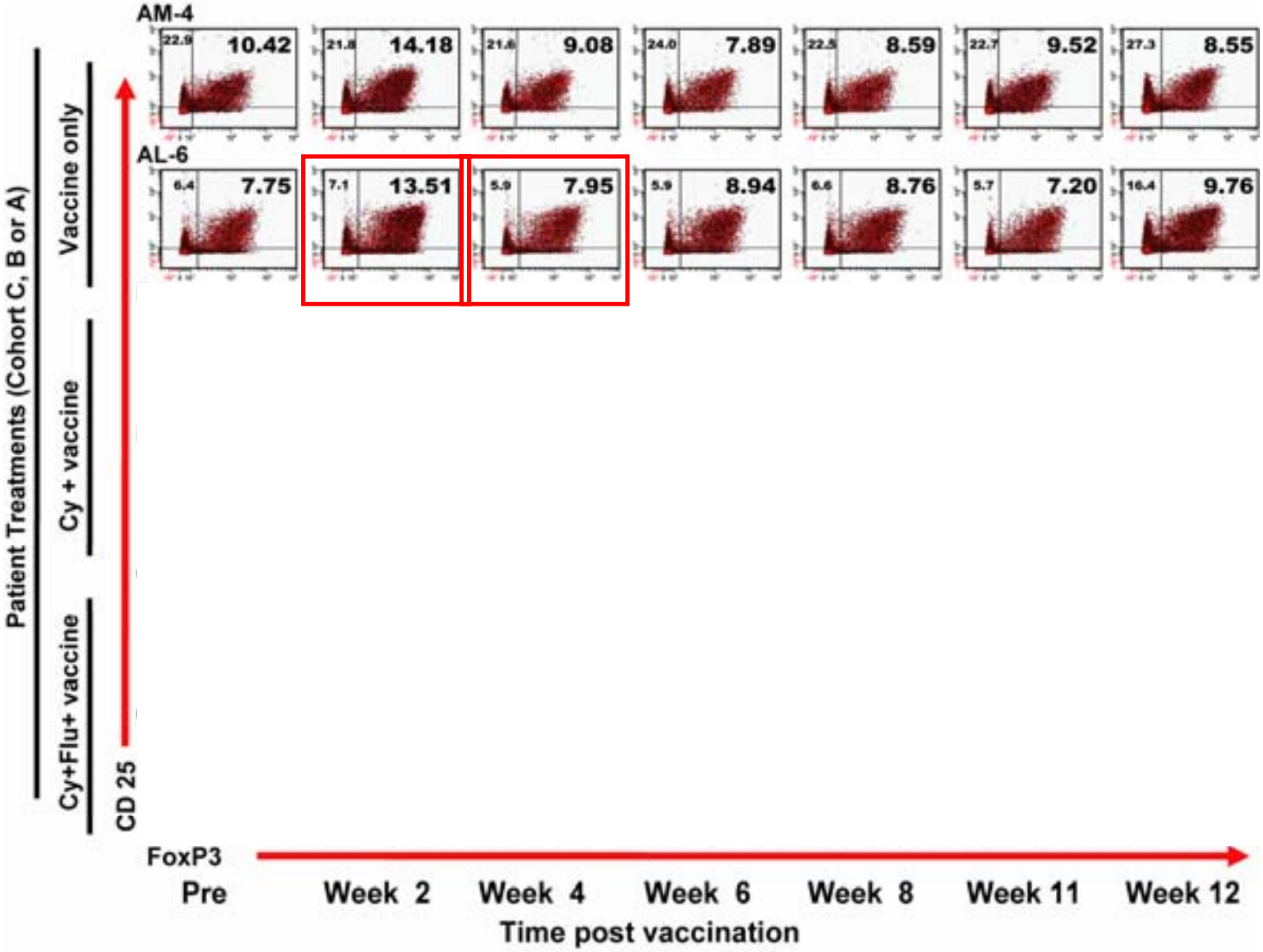
Clinical Trial Design

1) No chemotherapy / no reconstitution
2) Cyclophosphamide 350 mg/m^2 d 1-3
3) Cy + Fludarabine 20 mg/m^2 d 1-3



Do T_{reg} ($CD3^+/CD4^+/CD25^+/FoxP3^+$) Decrease After Reconstitution and Vaccination of Lymphopenic Patients?

T_{reg} ($CD3^+/CD4^+/CD25^+/FoxP3^+$) Increase After Vaccination.



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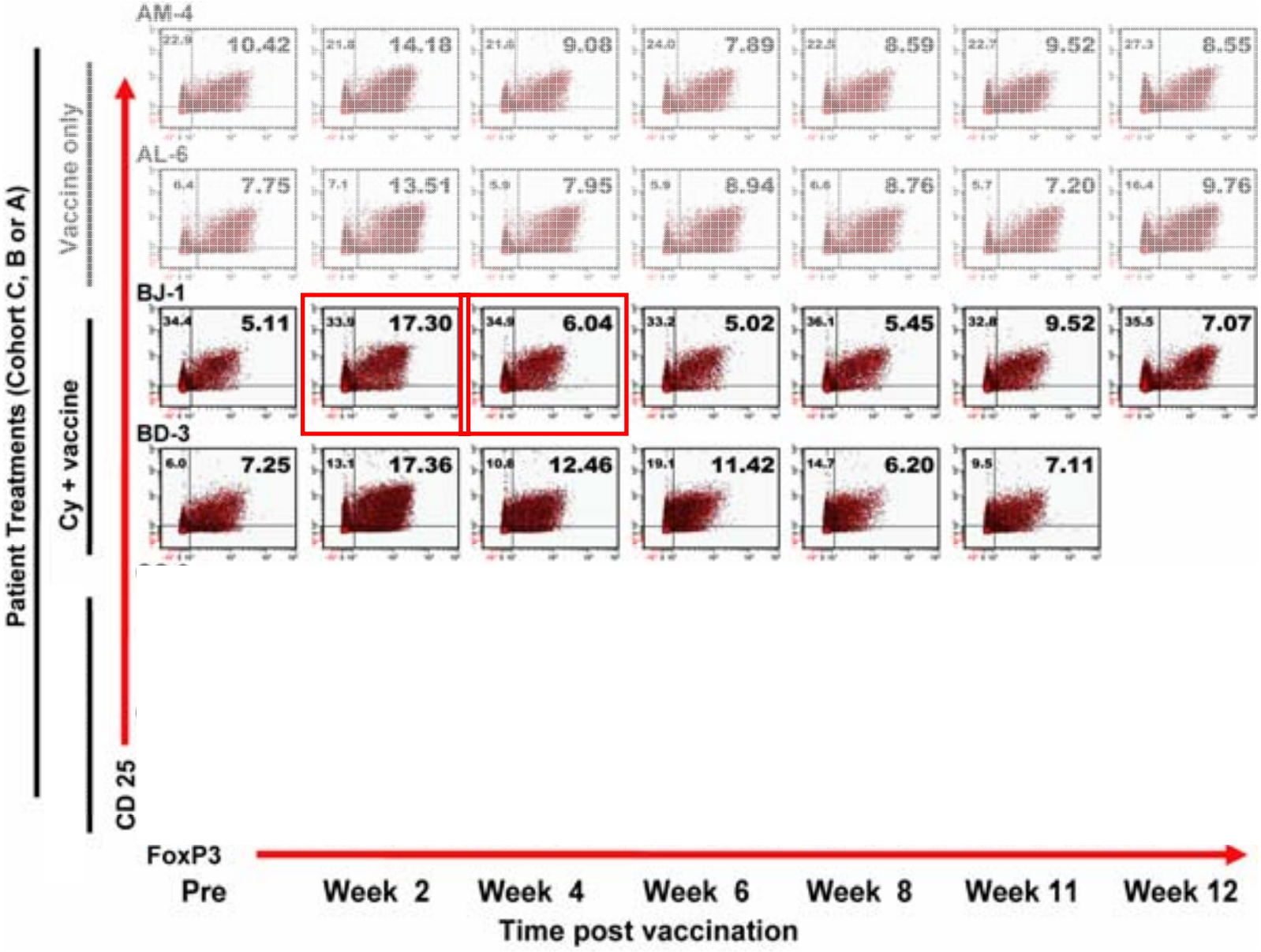

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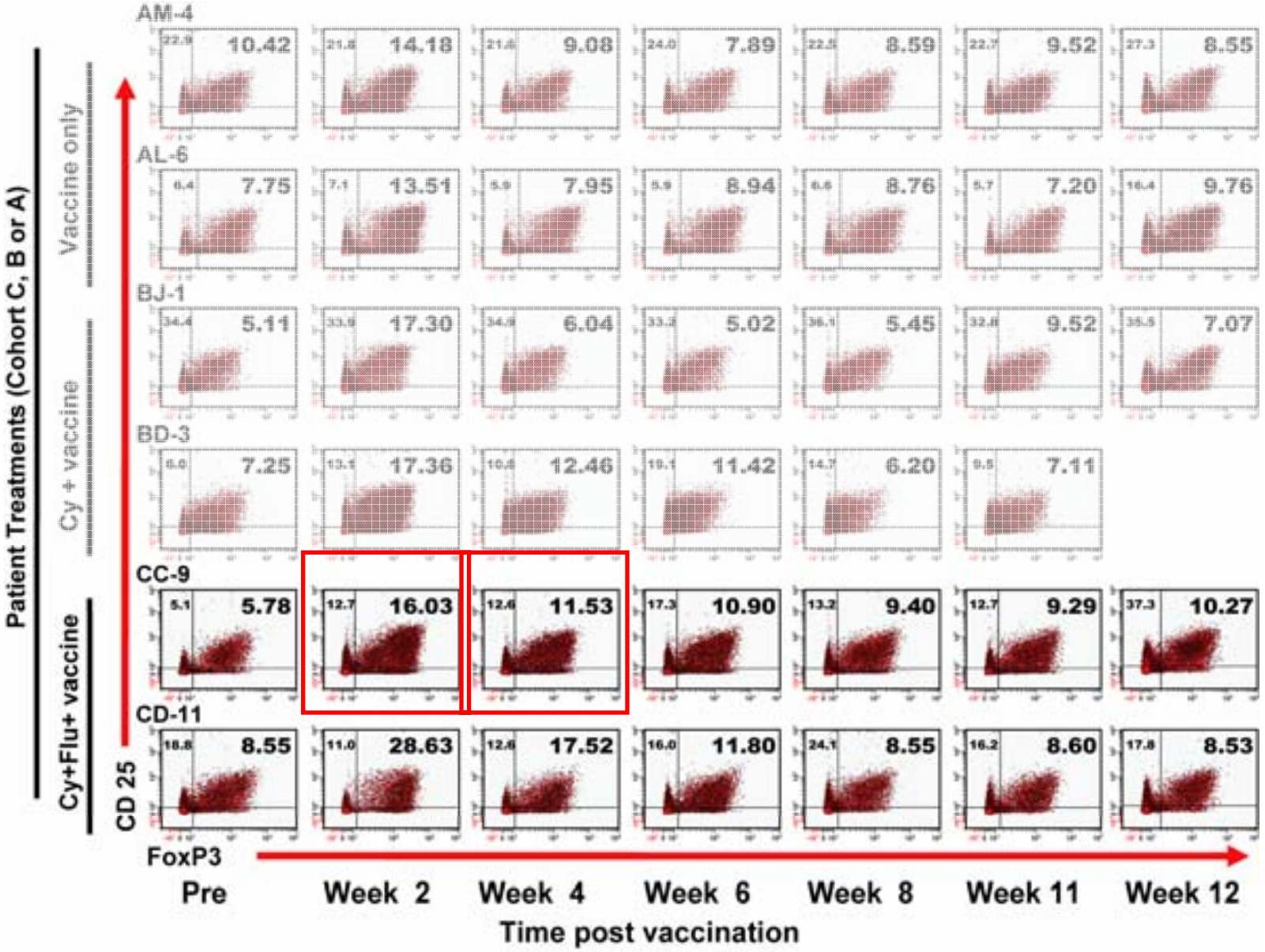

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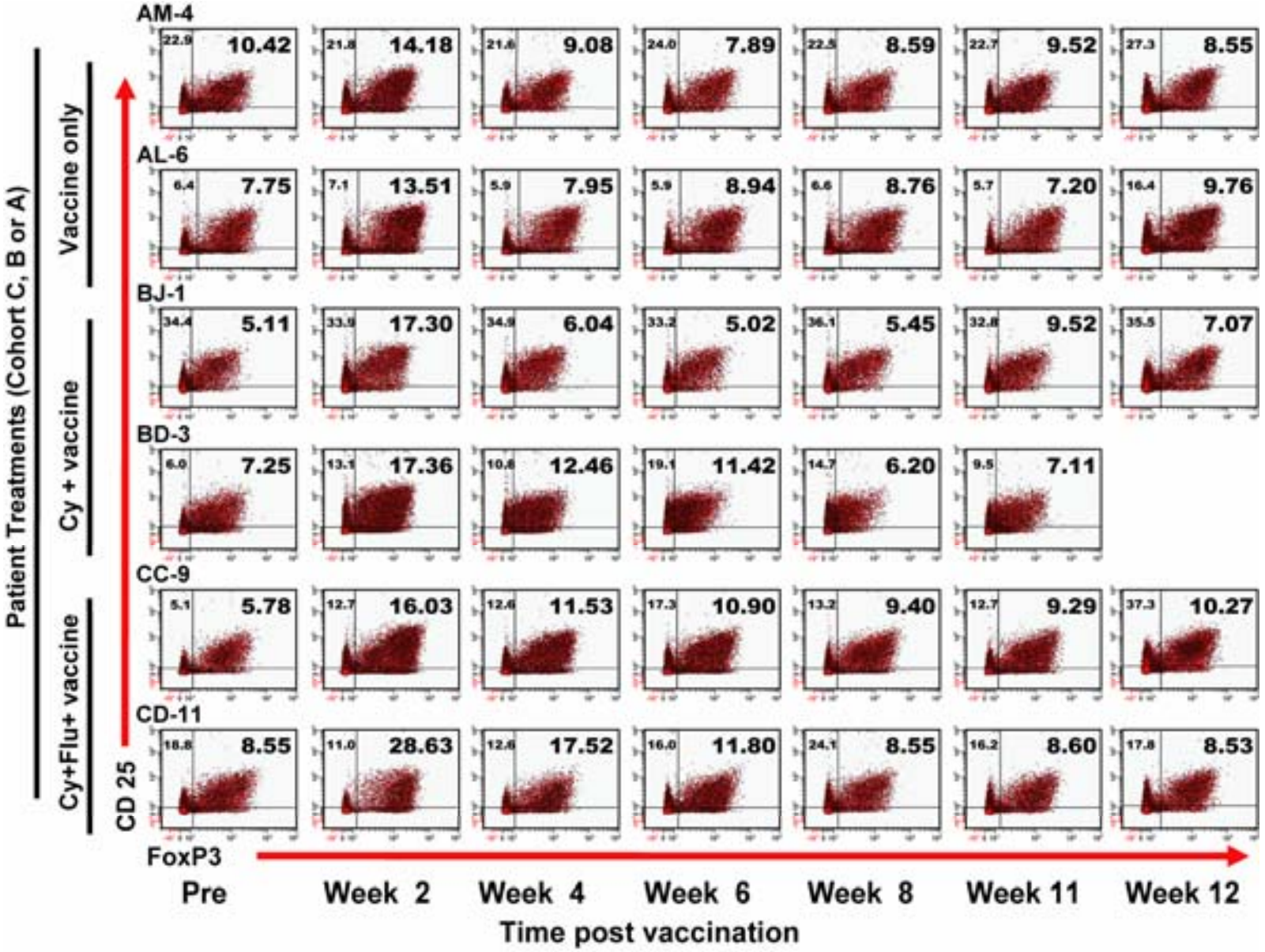

Are T_{reg} ($CD3^+/CD4^+/CD25^+/FoxP3^+$) dividing or entering the blood from the periphery?

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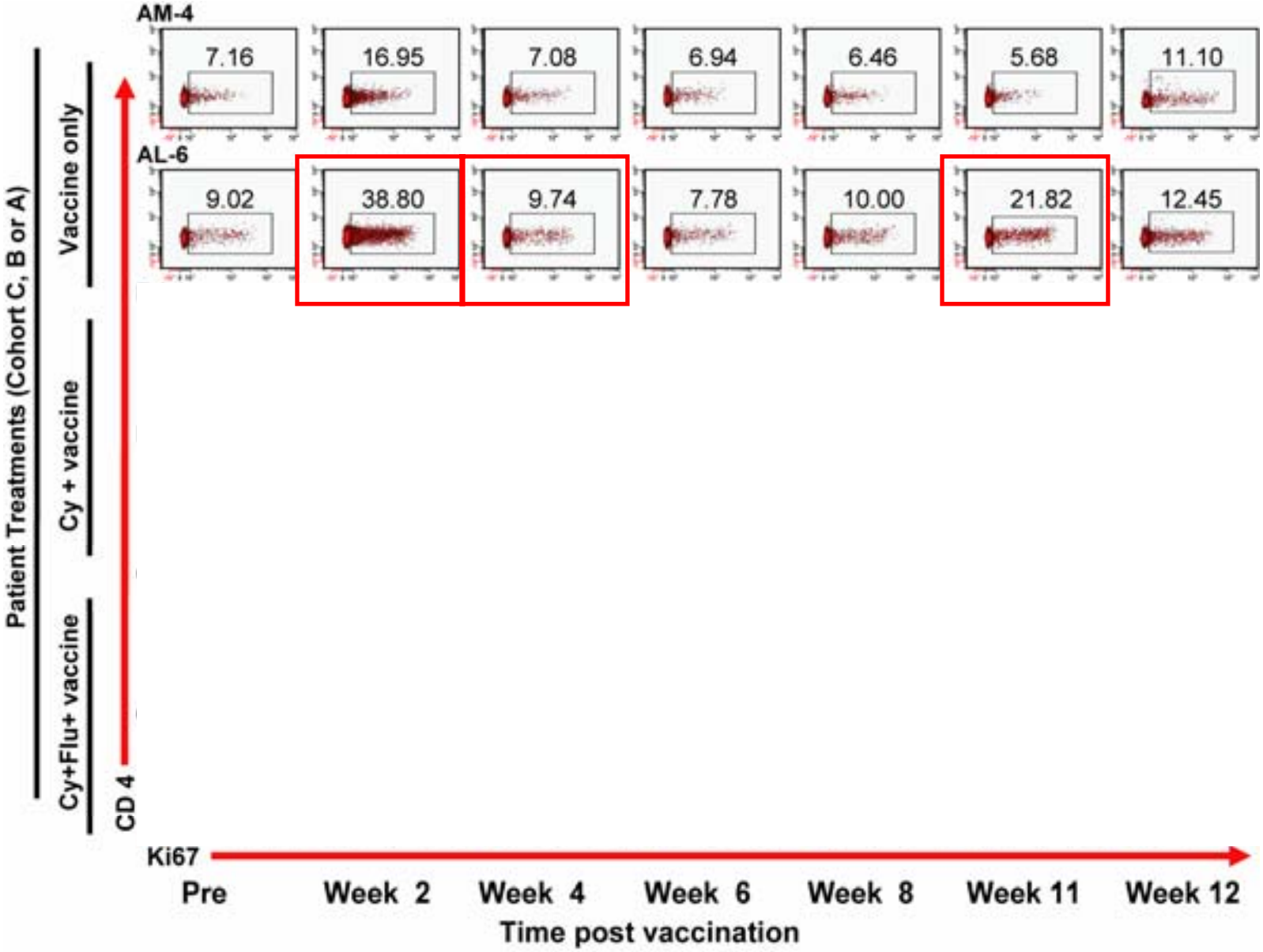

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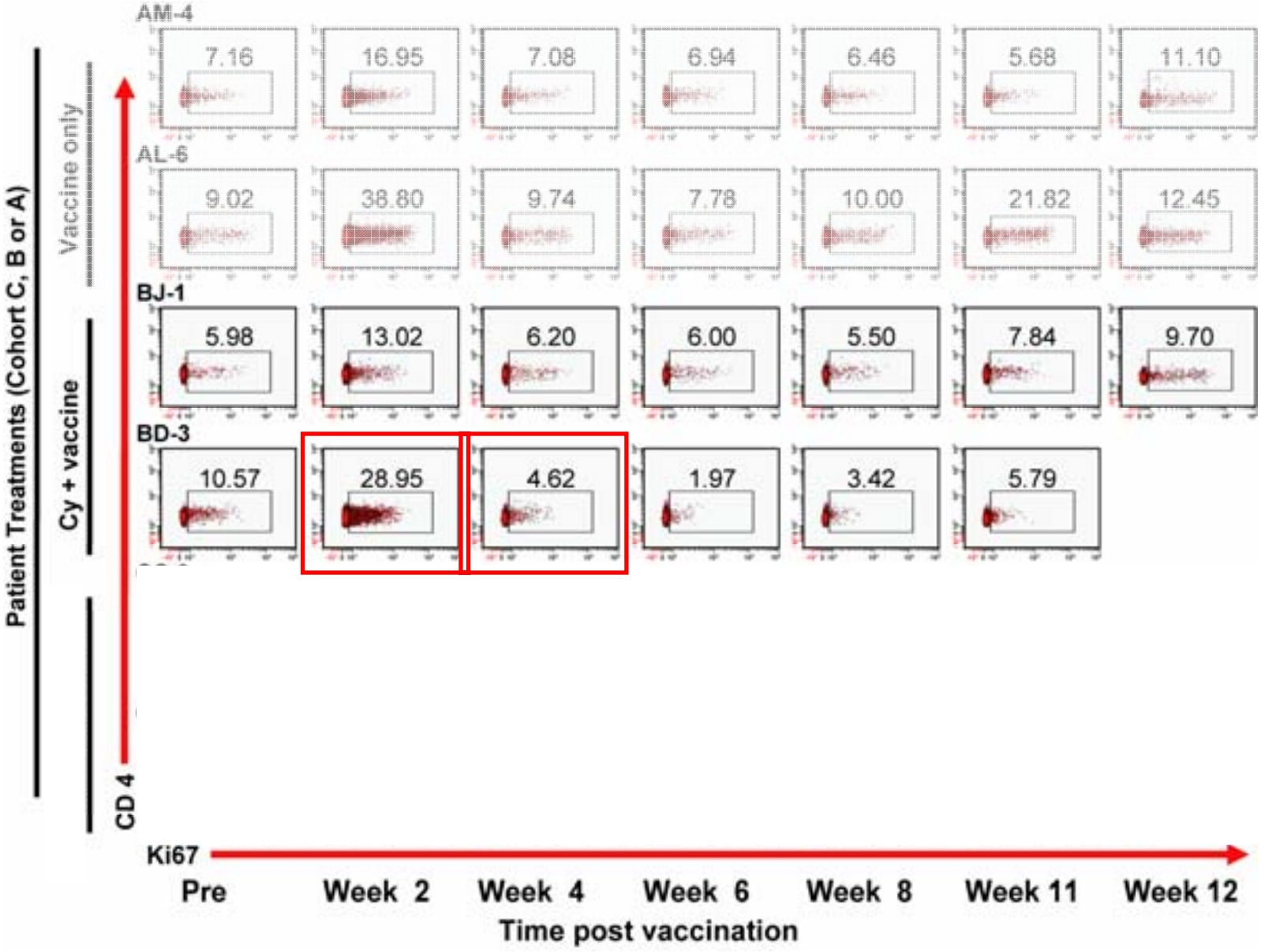

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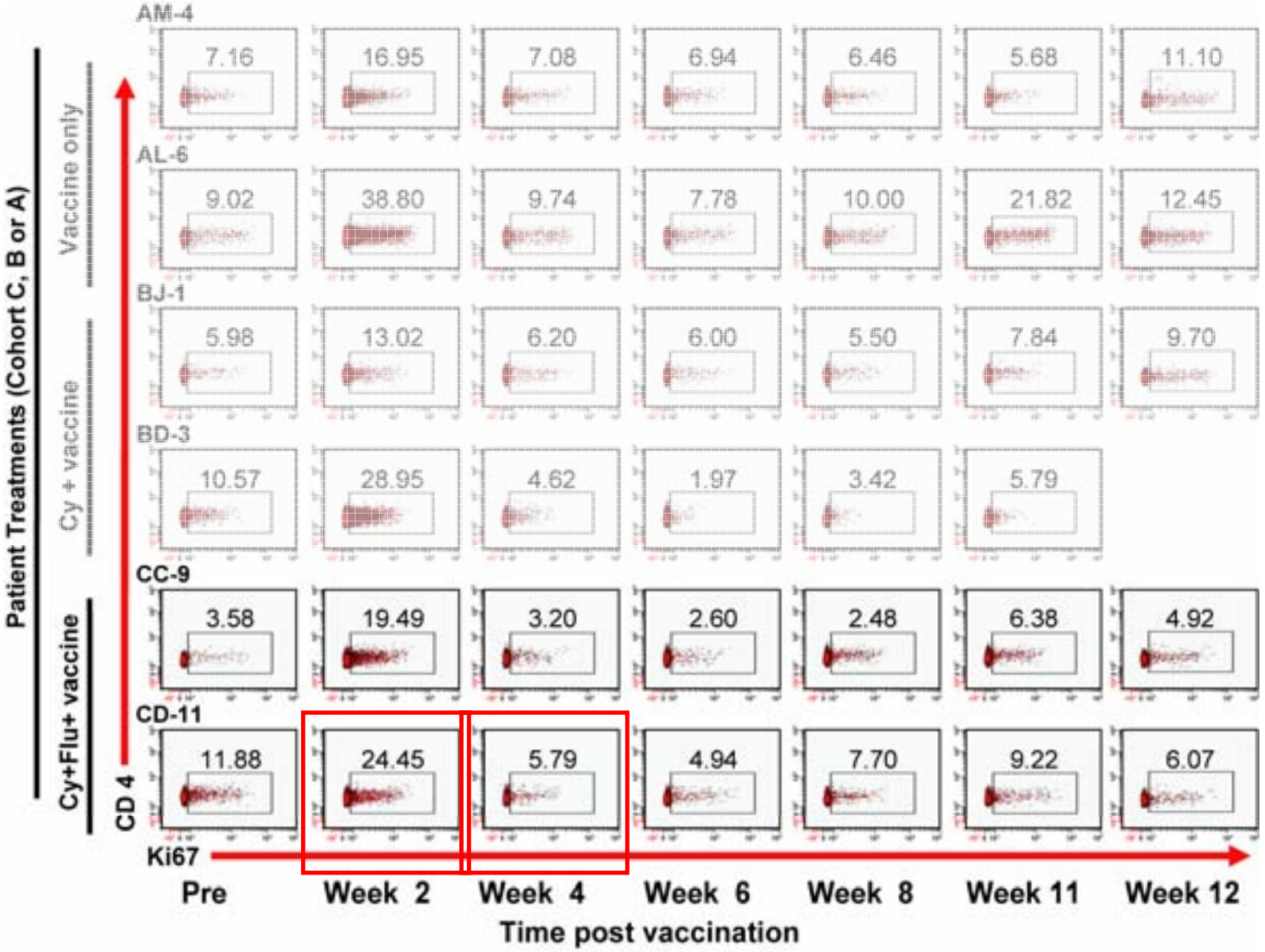

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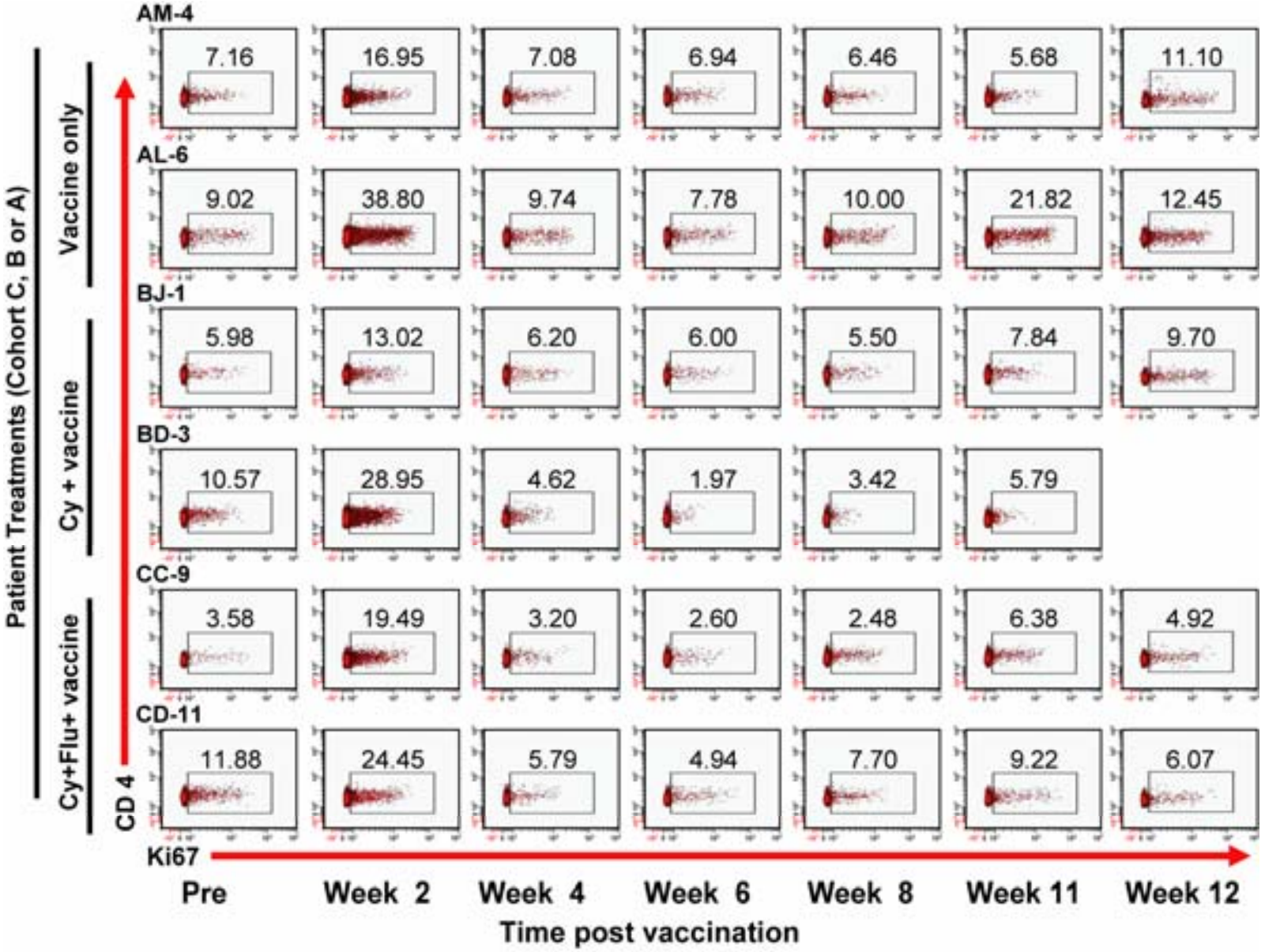
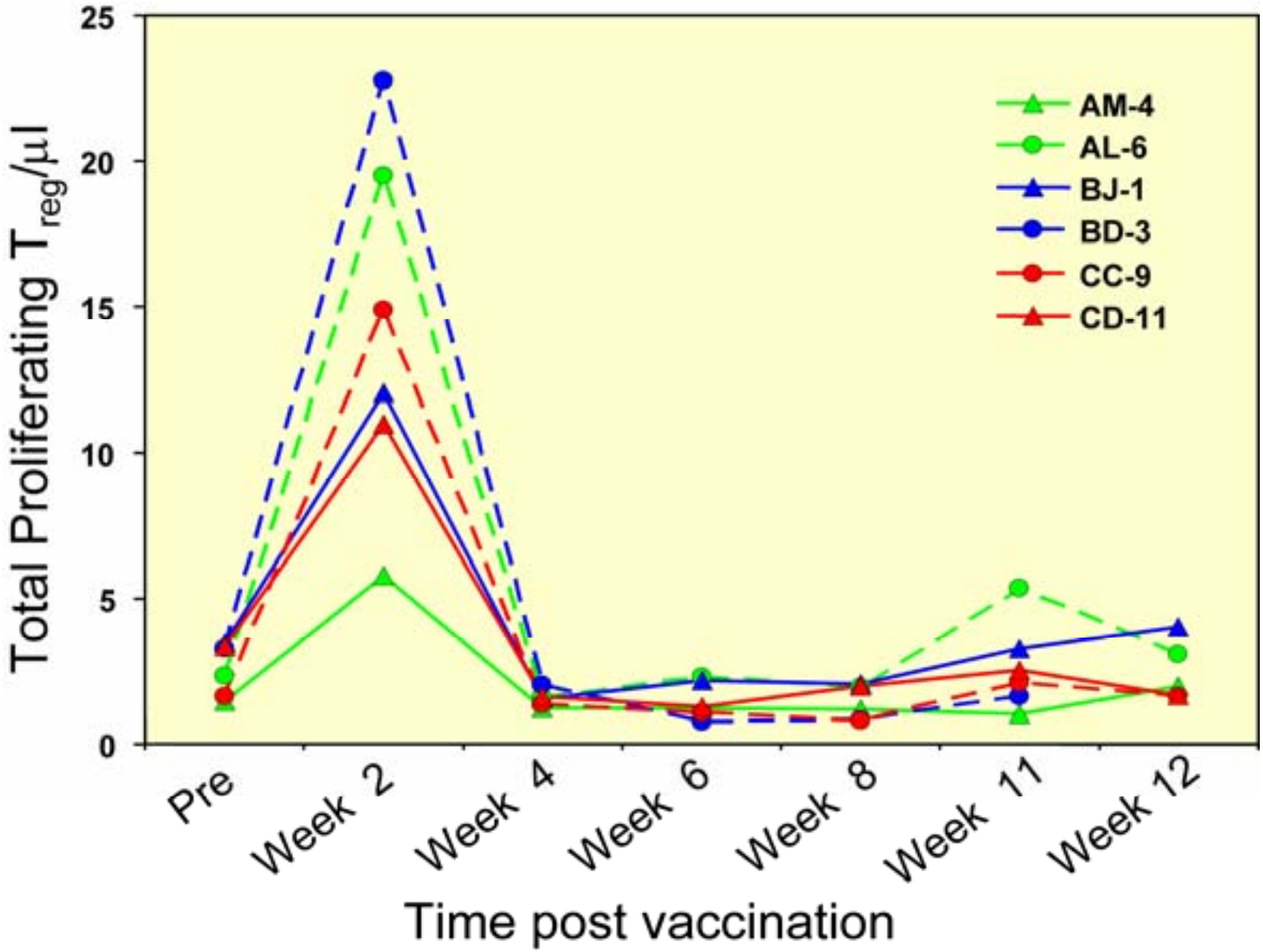


Are total numbers of dividing T_{reg} ($CD3^+/CD4^+/CD25^+/FoxP3^+$) increasing?



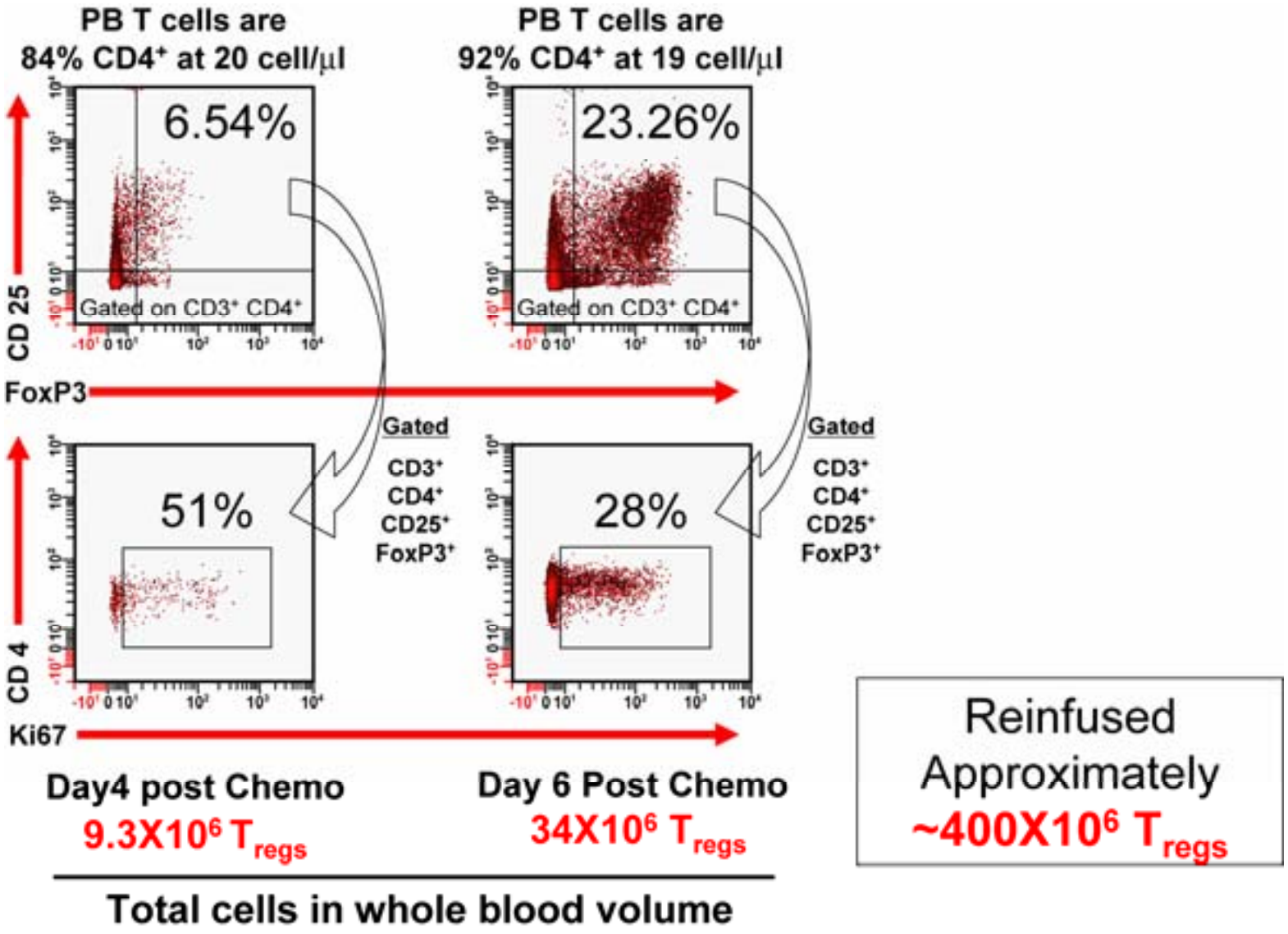
Question:

- Were the Treg resistant to the chemotherapy?

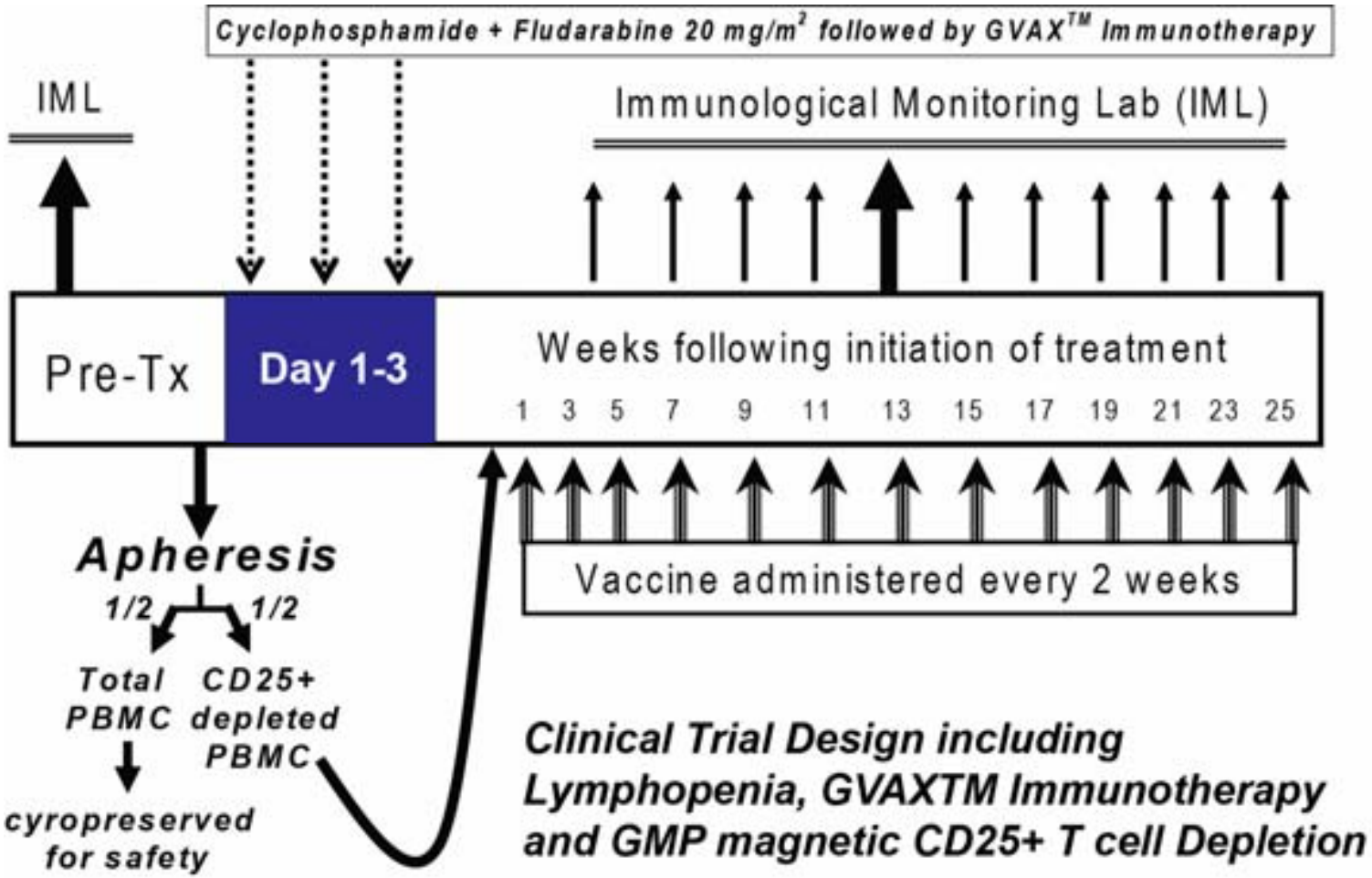
Or

- Did the adoptive transfer of PBMC “reconstitute” the Treg pool?

The majority of T_{reg} ($CD3^+/CD4^+/CD25^+/FoxP3^+$) come from the reinfusion product.



IRB-approved Clinical Trial



Are CD8+ T cells dividing in response to allogeneic prostate GVAX™ ?

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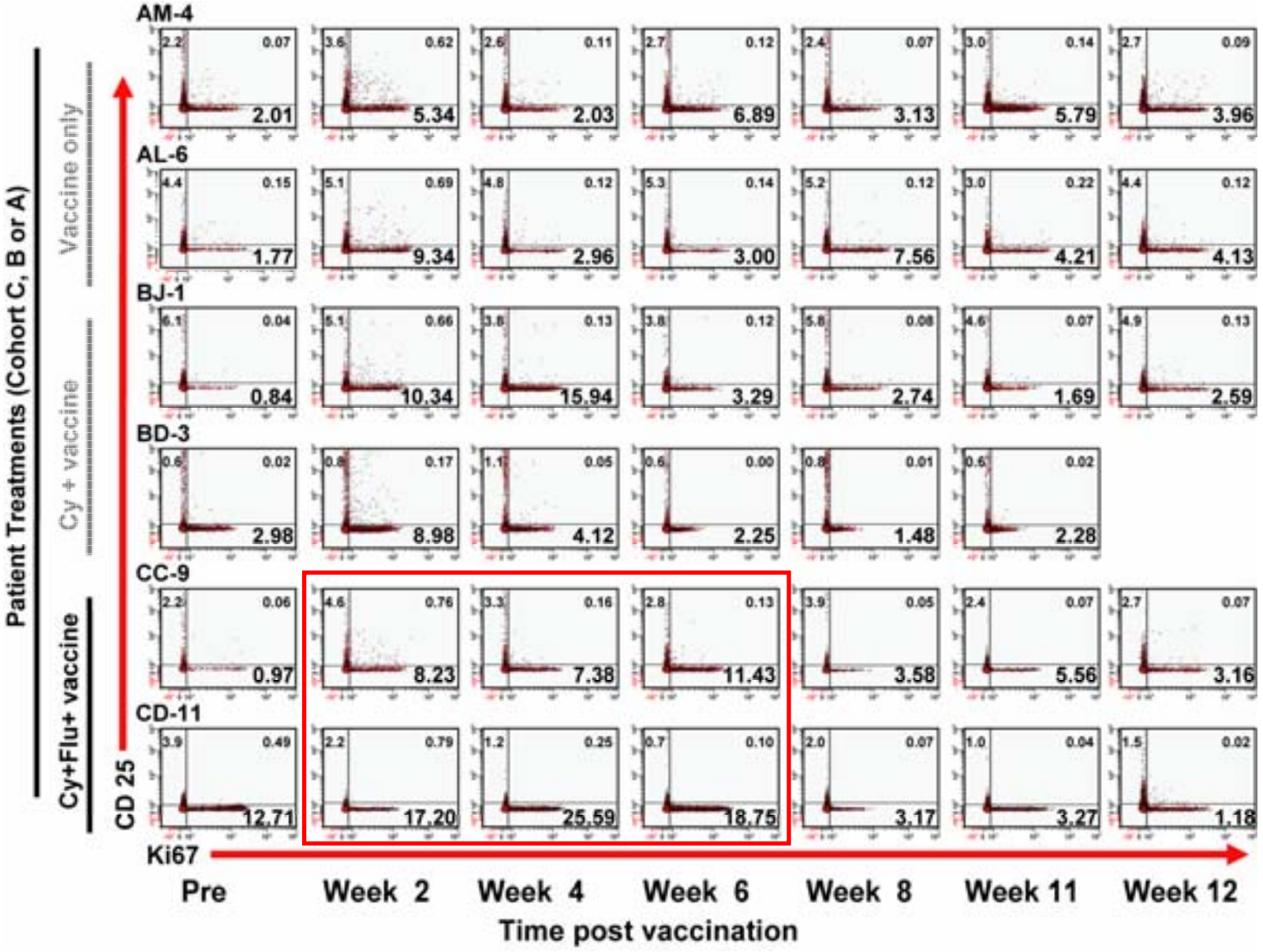
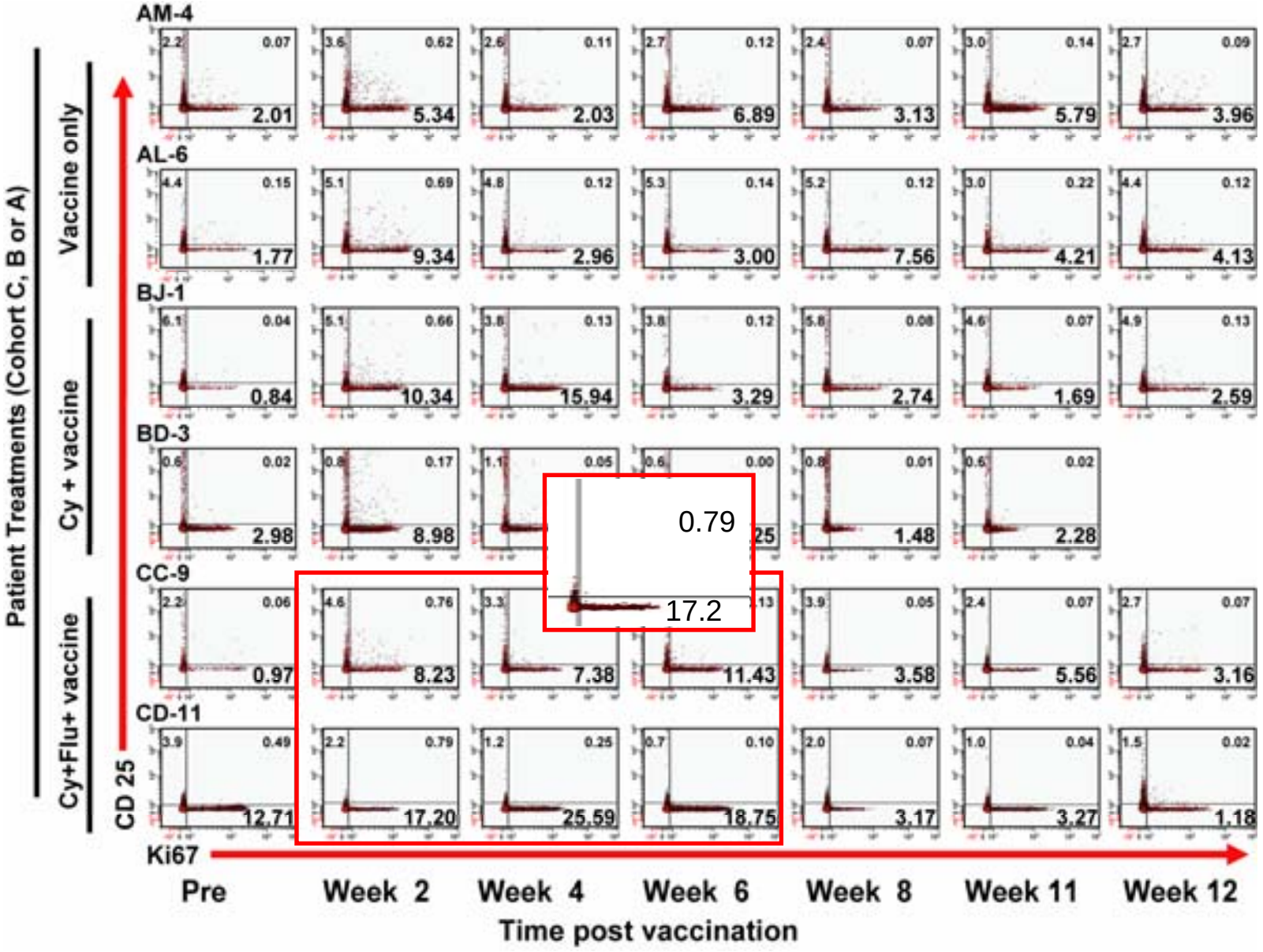


Are CD8+ T cells dividing in response to allogeneic prostate GVAX™ ?



Summary and Conclusions

1. Prostate GVAX immunotherapy can be administered safely to patients receiving cyclophosphamide and fludarabine and reconstituted with PBMC.

2. Unexpectedly, The frequency of CD3+CD4+CD25+FoxP3+Treg cells is increased two weeks following vaccination and the increase appears to be maintained longer in patients receiving chemotherapy. Similar findings were observed following a NSCLC vaccine (iSBTc Poster, Natasja van den Engel, LMU Munich, Germany) .

3. CD4+FoxP3+ and CD8+FoxP3- T cells divide in response to Allo Prostate GVAX™.
   - CD4+FoxP3+ proliferation peaks at week 2.
   - Dividing CD8+FoxP3- T cells are CD25 negative.

4. Preliminary data suggests that a majority of the FoxP3+ T cells present in the peripheral blood of cohorts B and C are derived from the infused PBMCs.

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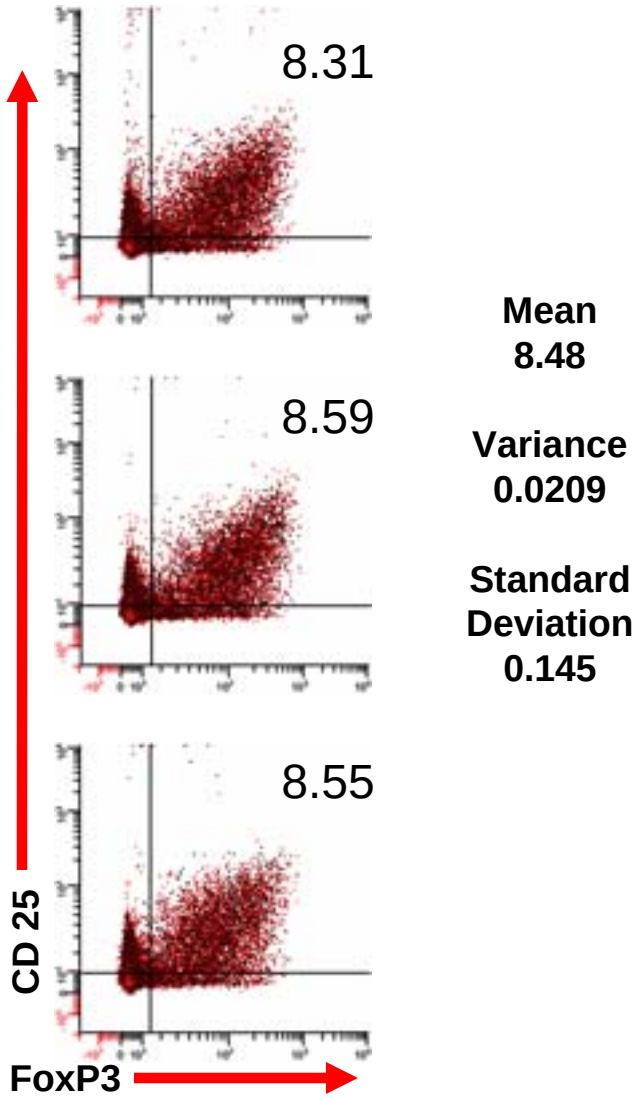
Are differences seen in T_{reg} staining ($CD3^+$, $CD4^+$, $CD25^+$, $FoxP3^+$) and Proliferation assays ($Ki67^+$) significant?

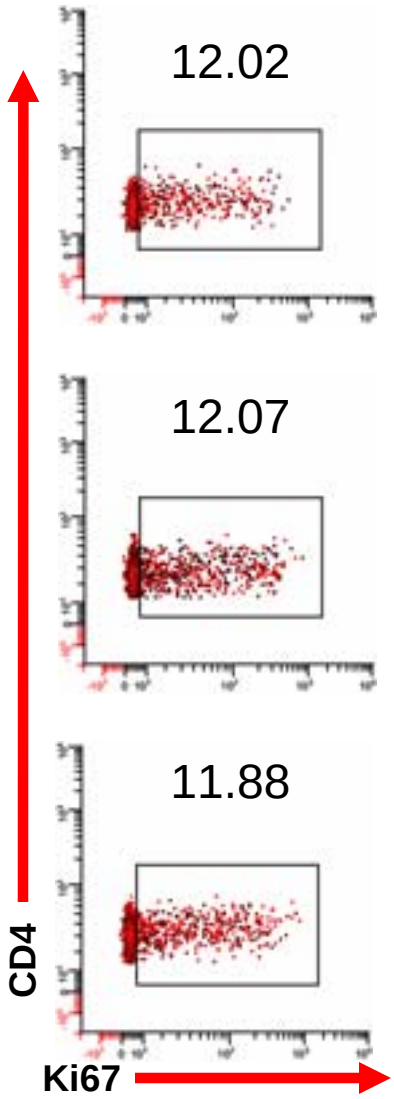


Mean
8.48

Variance
0.0209

Standard Deviation
0.145

Gated on $CD3^+$ $CD4^+$ Cells



Mean
11.99

Variance
0.0065

Standard Deviation
0.08

Gated on $CD3^+$ $CD4^+$ $CD25^+$ $FoxP3^+$ Cells