



# SITC 2017

November 8-12  
NATIONAL HARBOR  
MARYLAND

Gaylord National Hotel  
& Convention Center



Society for Immunotherapy of Cancer

November 8-12 • NATIONAL HARBOR, MD

SITC  
2017



INSTITUTE FOR  
INFECTION &  
IMMUNITY

**ICVI** Institute for  
Cancer Vaccines  
& Immunotherapy  
Changing Lives Through Research  
icvi.org.uk

# Zoledronic acid induces $V\delta 2^+$ $\gamma\delta$ T cells to target macrophages

Dr Daniel Fowler  
Institute for Cancer Vaccines & Immunotherapy



Society for Immunotherapy of Cancer

#SITC2017

# Presenter Disclosure Information

*Daniel Fowler*

The following relationships exist related to this presentation:

*No Relationships to Disclose*



# Background

## □ Vδ2<sup>+</sup> γδ T cells

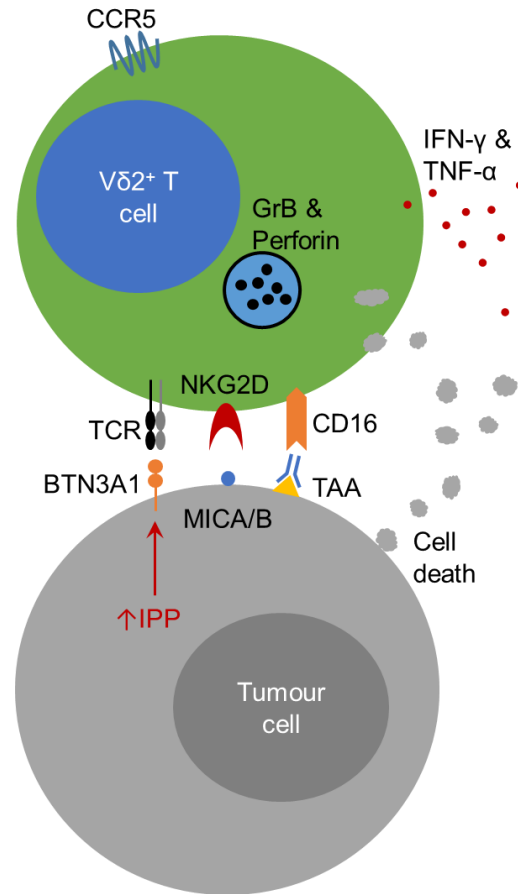
- Tissue homing memory cells
- Innate-like recognition of tumour
- Multiple effector functions

## □ Zoledronic acid (ZA)

- Increases tumour killing
- Blocks mevalonate pathway
- Cancer immunotherapy

## □ Macrophages (Mφs)

- Abundant in tumour
- Pro- or anti-tumour
- Take up ZA
- Does ZA render Mφs susceptible to Vδ2<sup>+</sup> T cell cytotoxicity?



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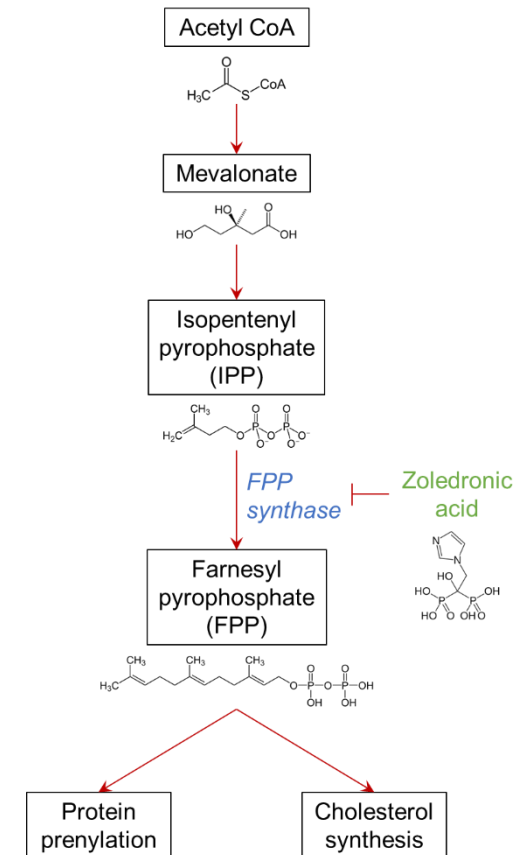
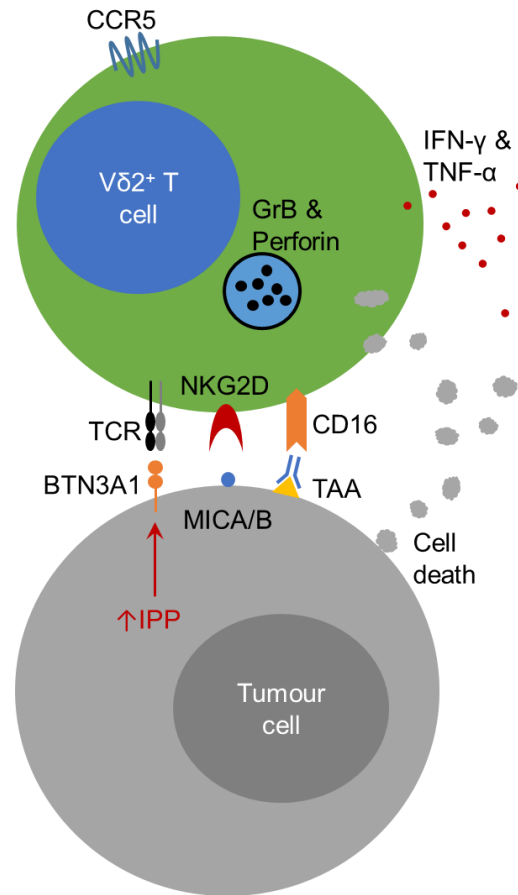
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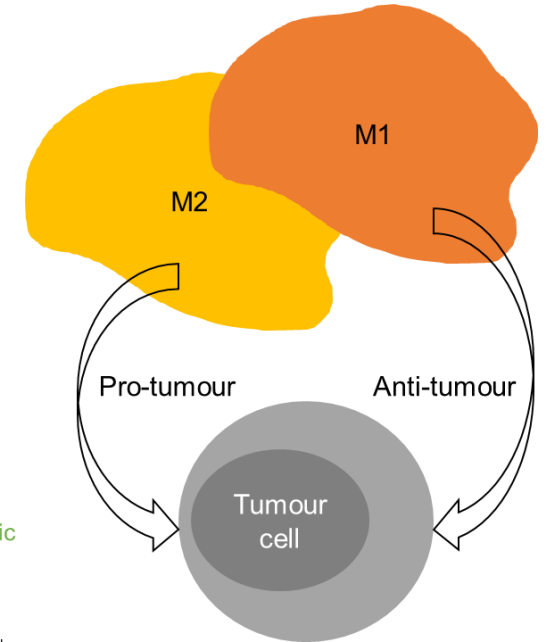
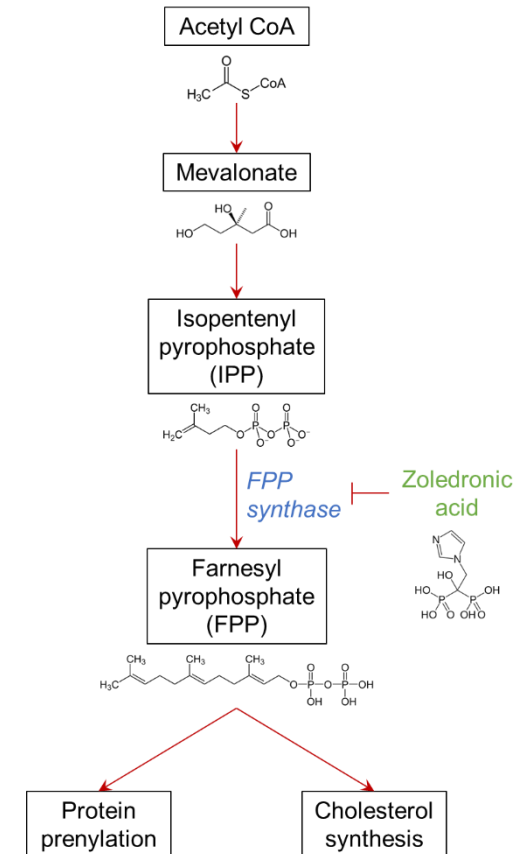
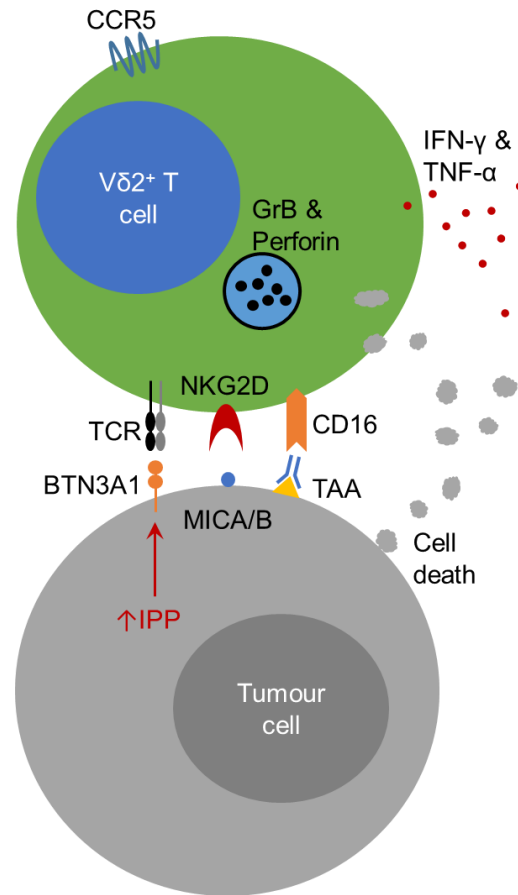
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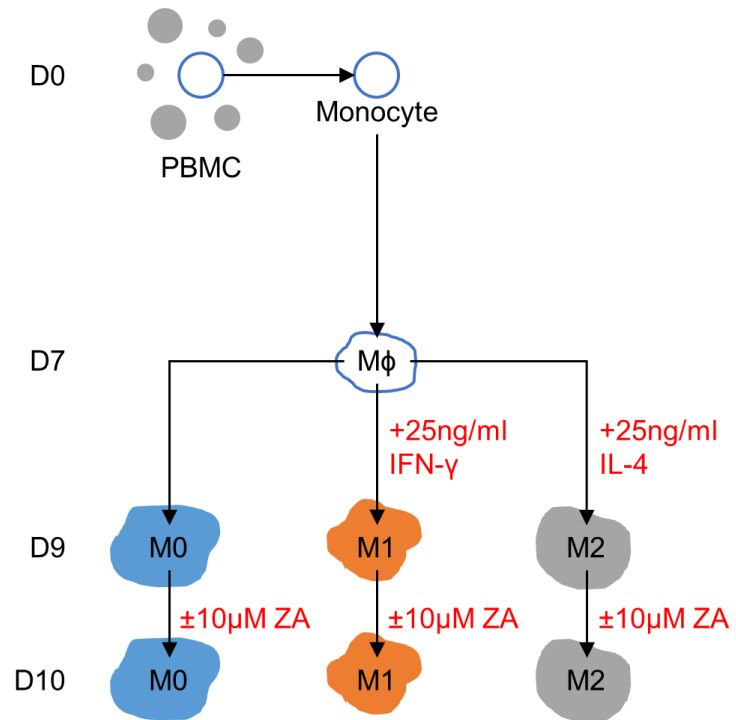
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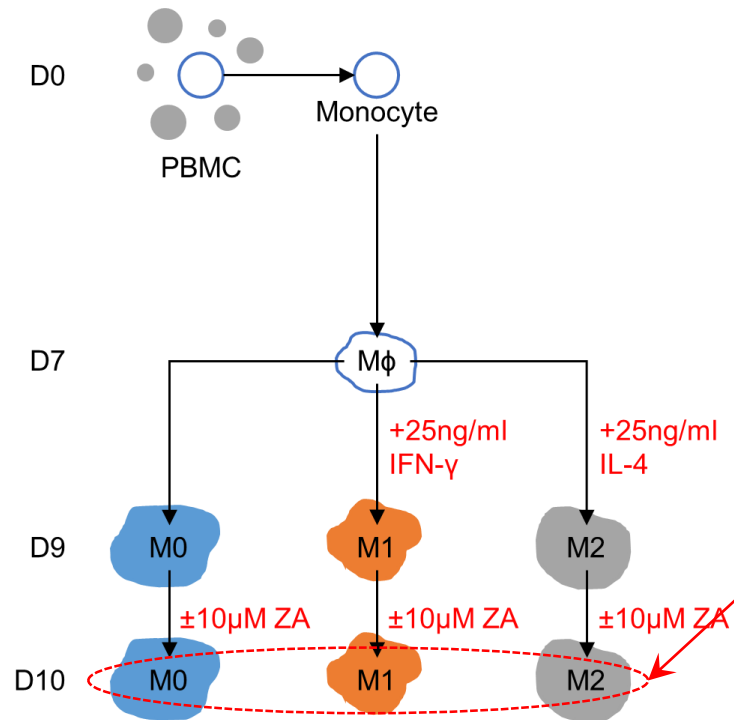
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# Monocyte differentiation



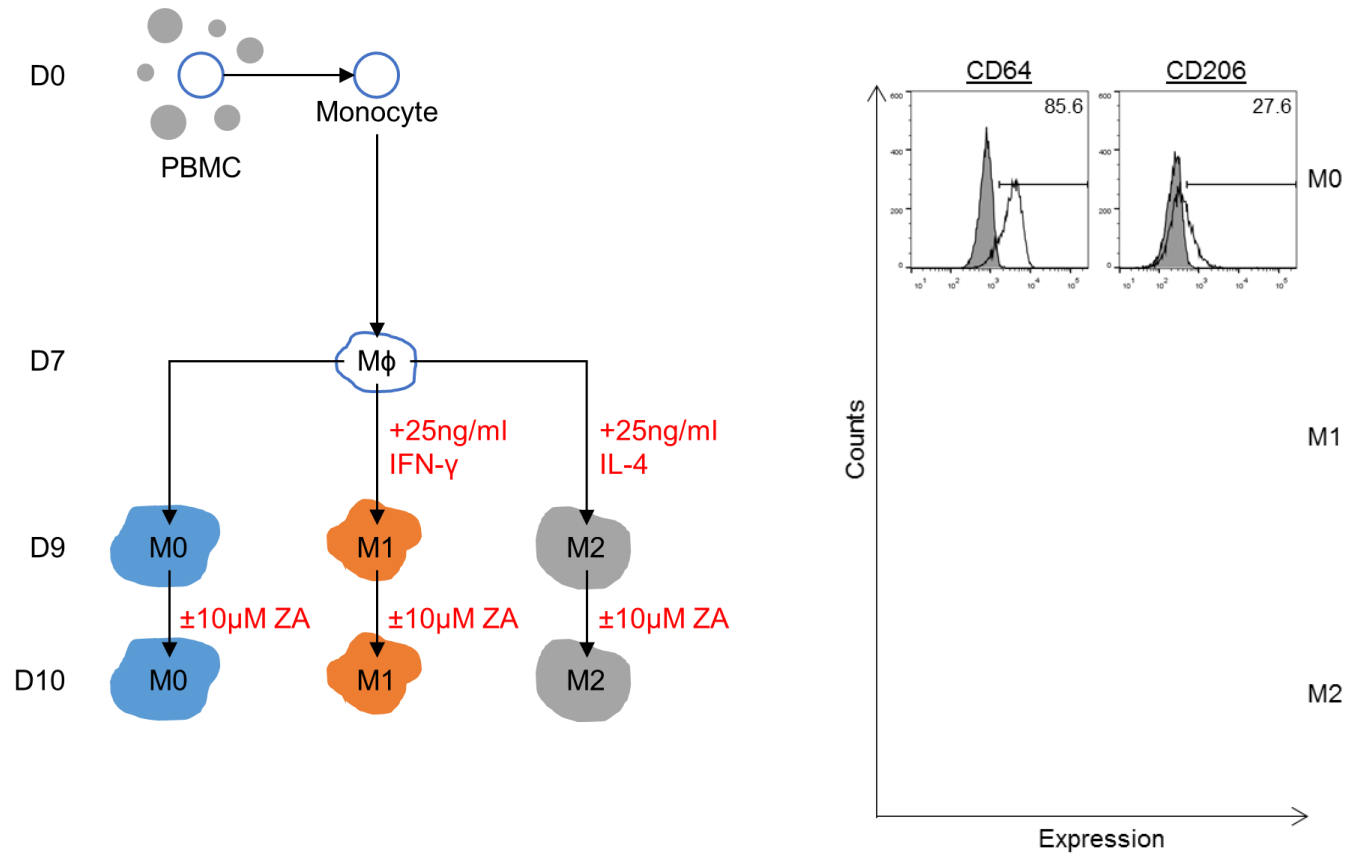
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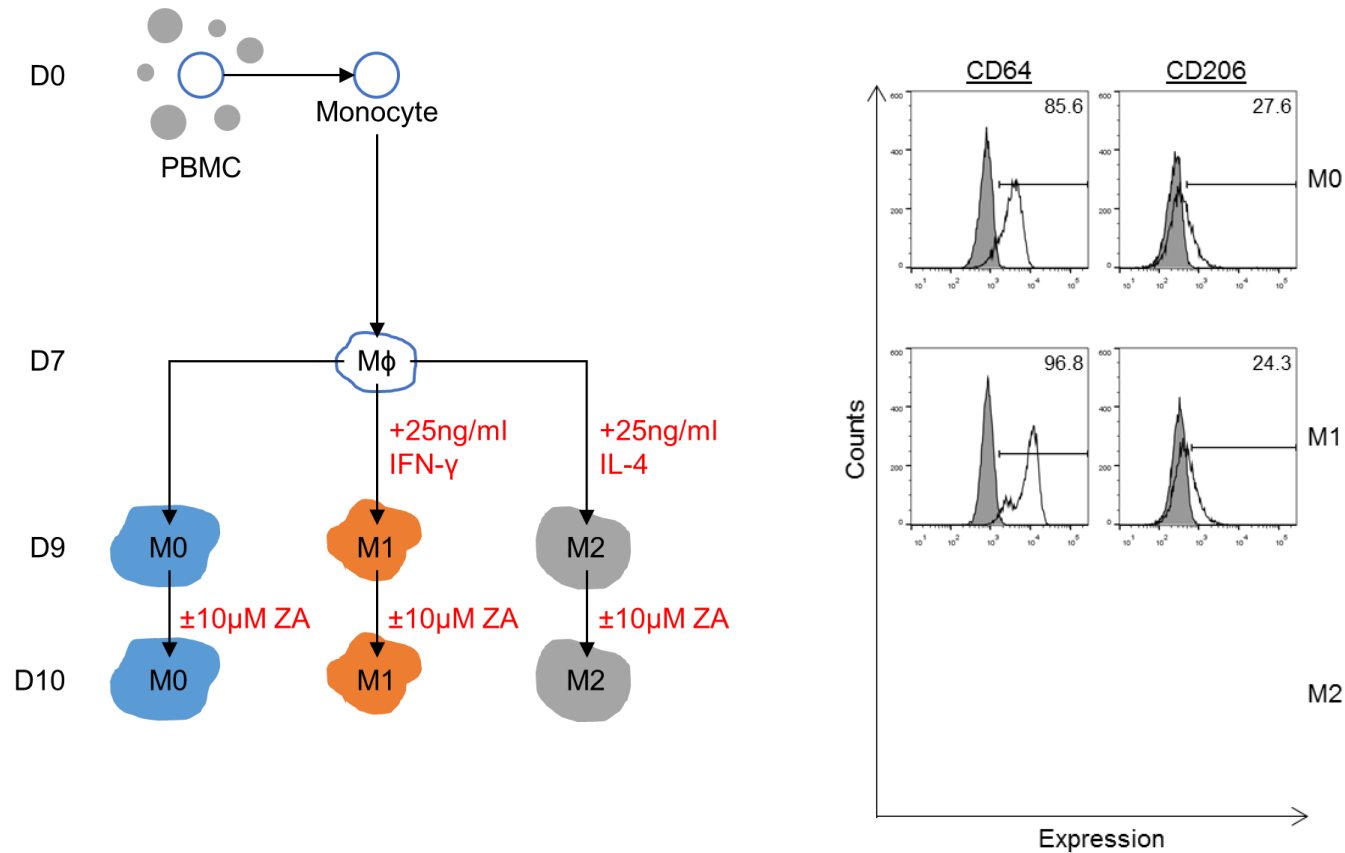
Flow cytometry to measure expression of the M1 & M2 markers CD64 & CD206



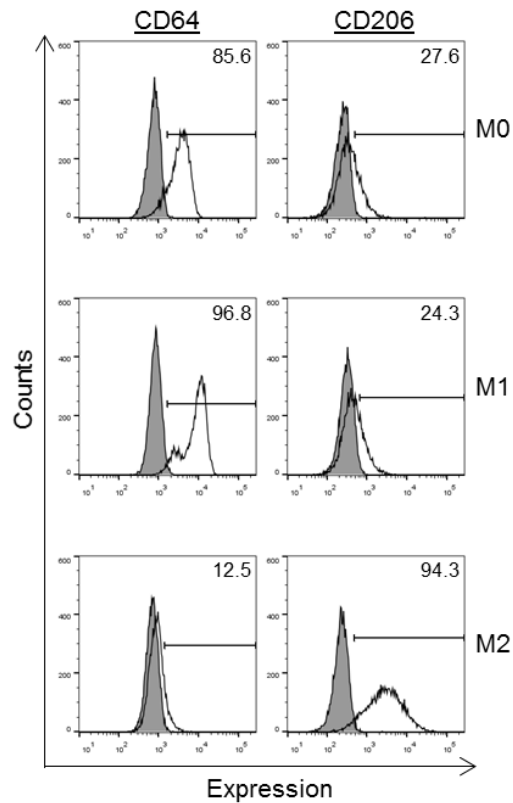
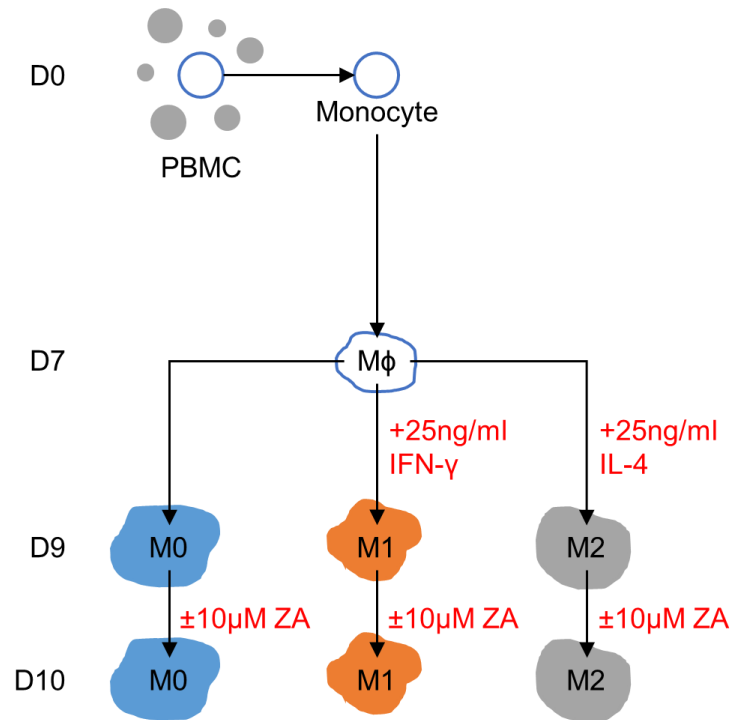
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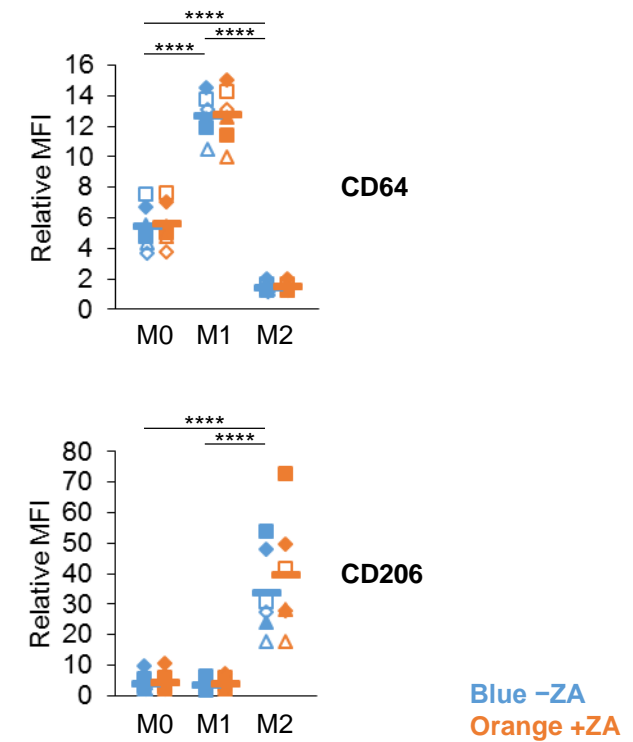
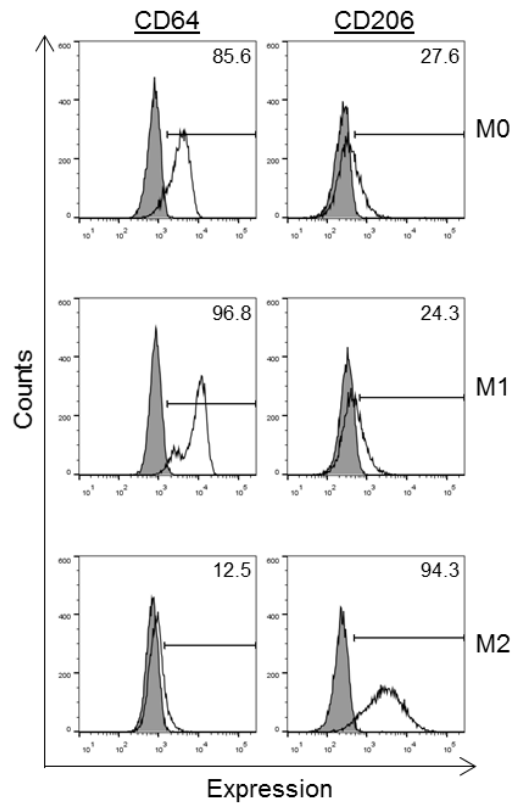
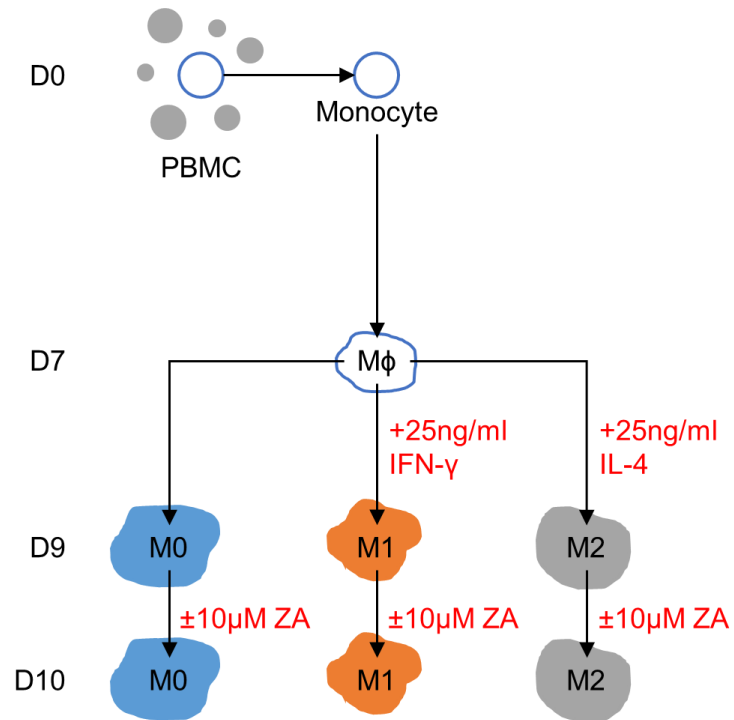
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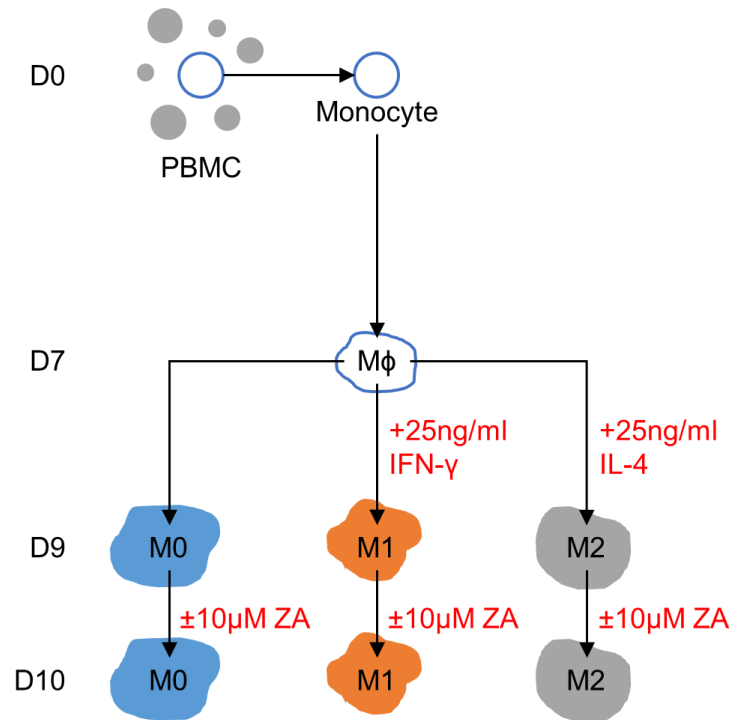
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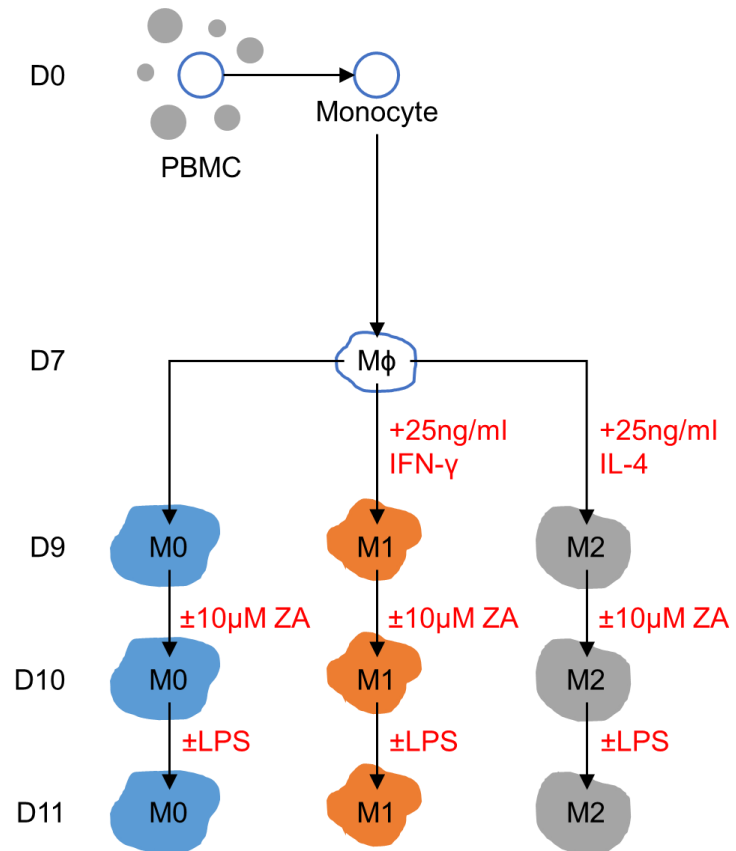


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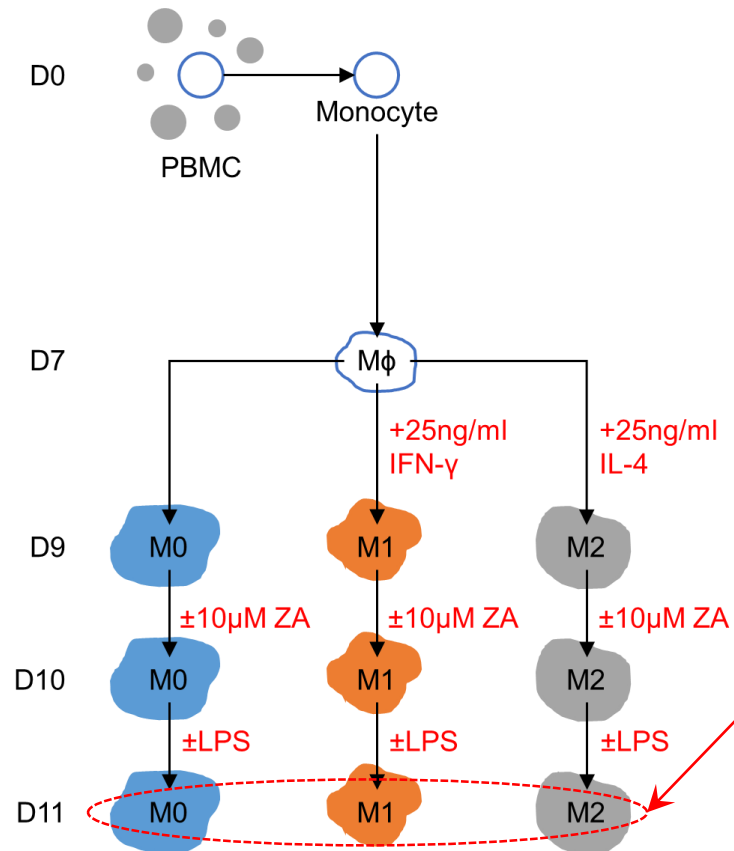




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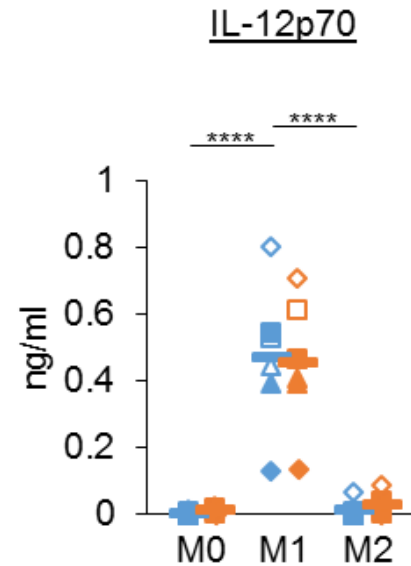
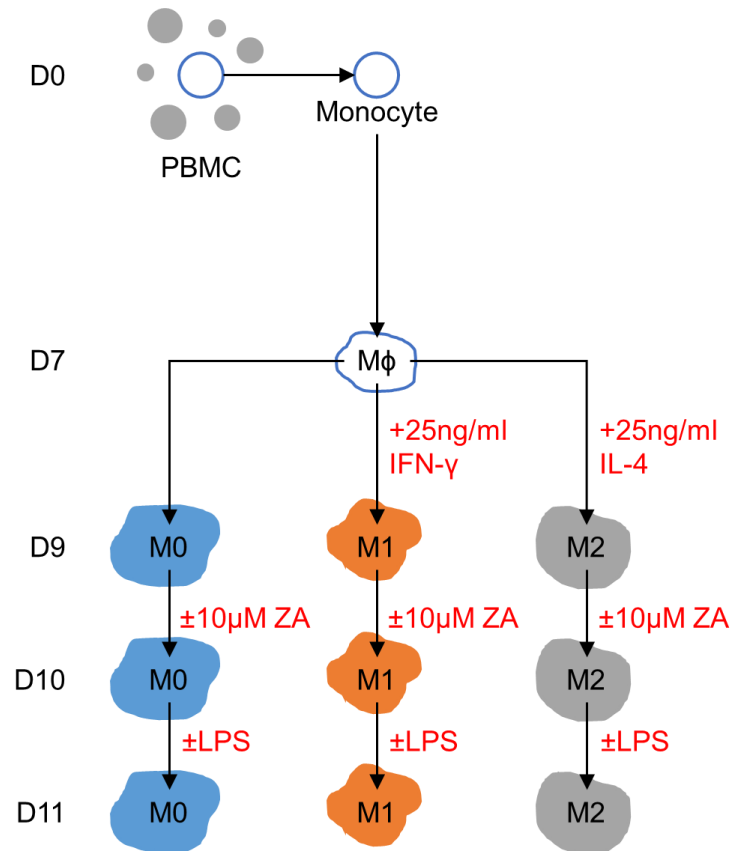


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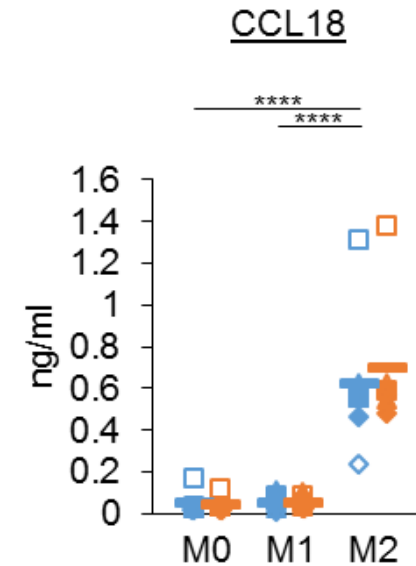
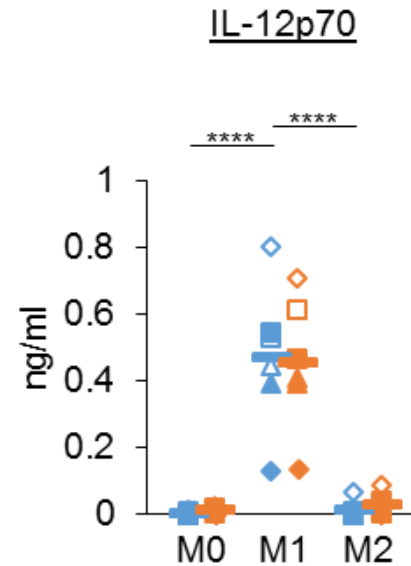
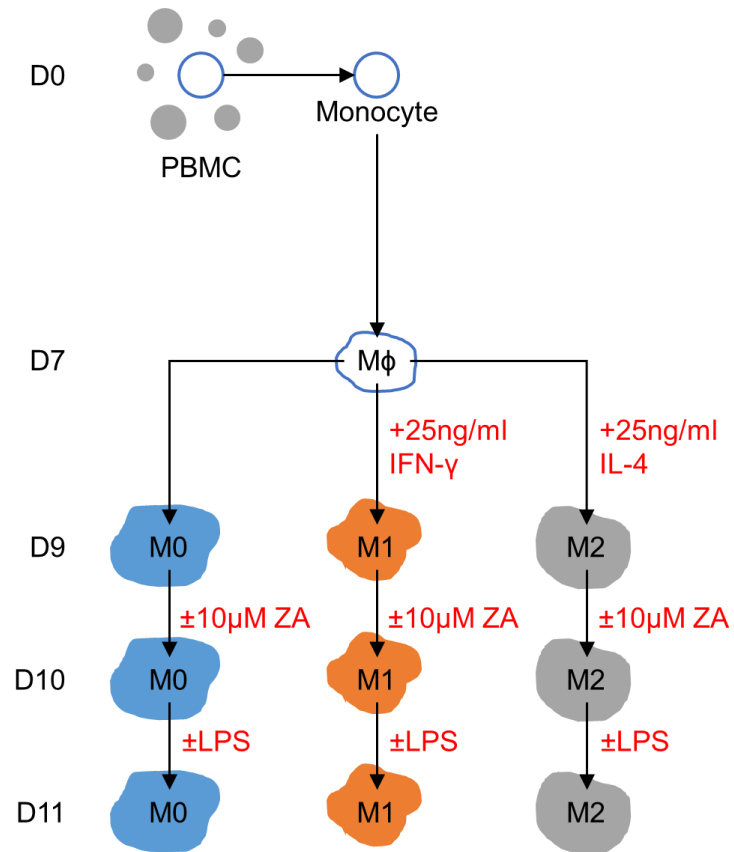
ELISA to measure production of  
the M1-related cytokine IL-12p70  
& the M2-related chemokine  
CCL18

# Monocyte differentiation



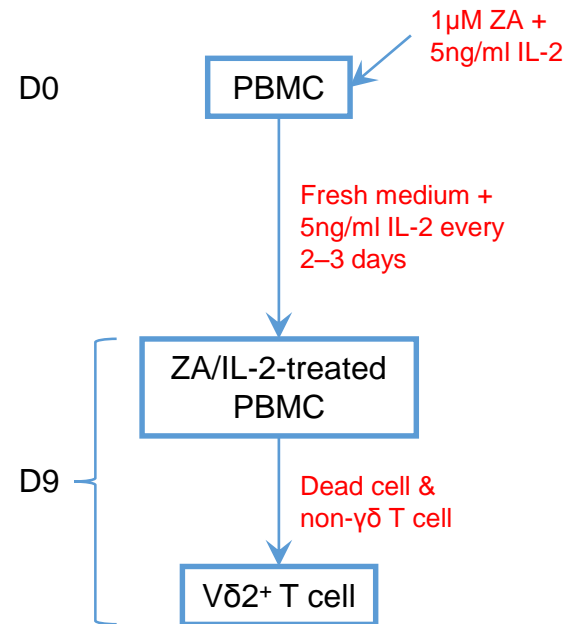
Blue -ZA  
Orange +ZA

# Monocyte differentiation



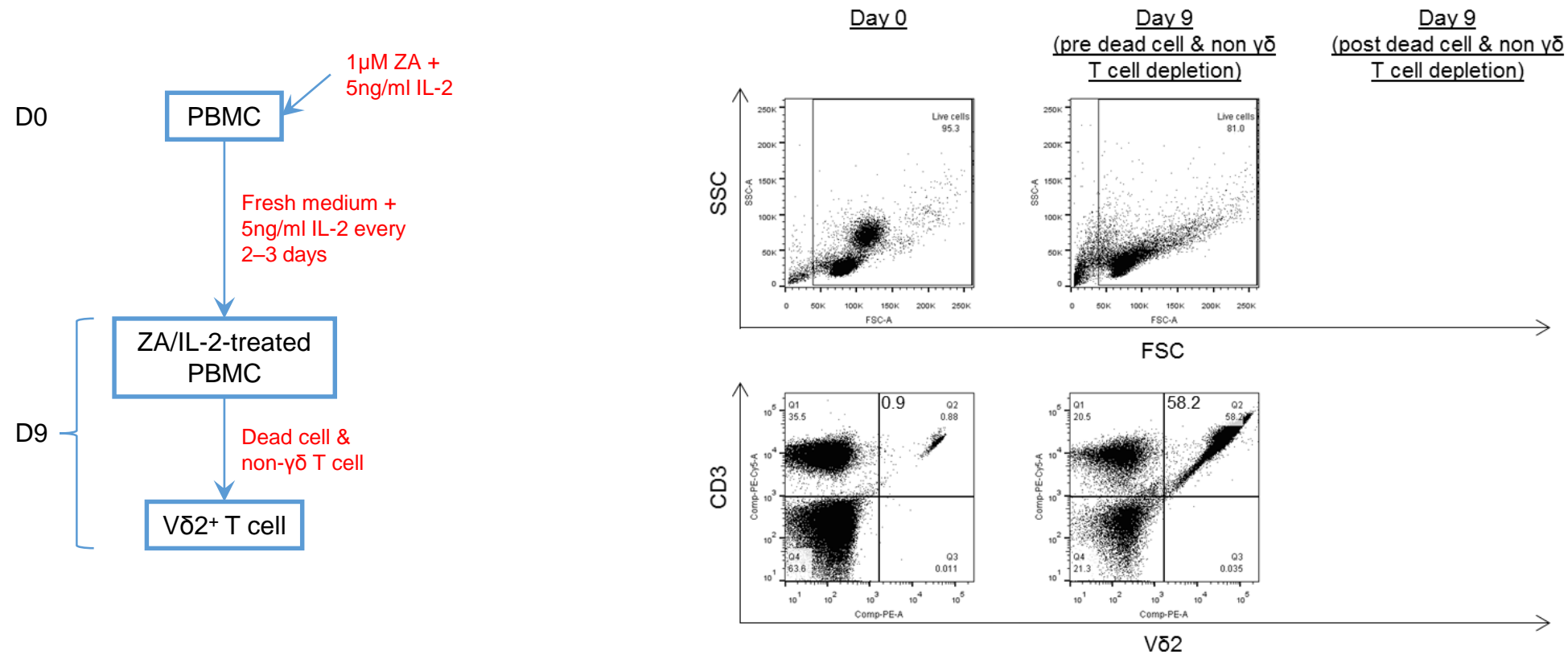
Blue -ZA  
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# V $\delta$ 2<sup>+</sup> T cell isolation

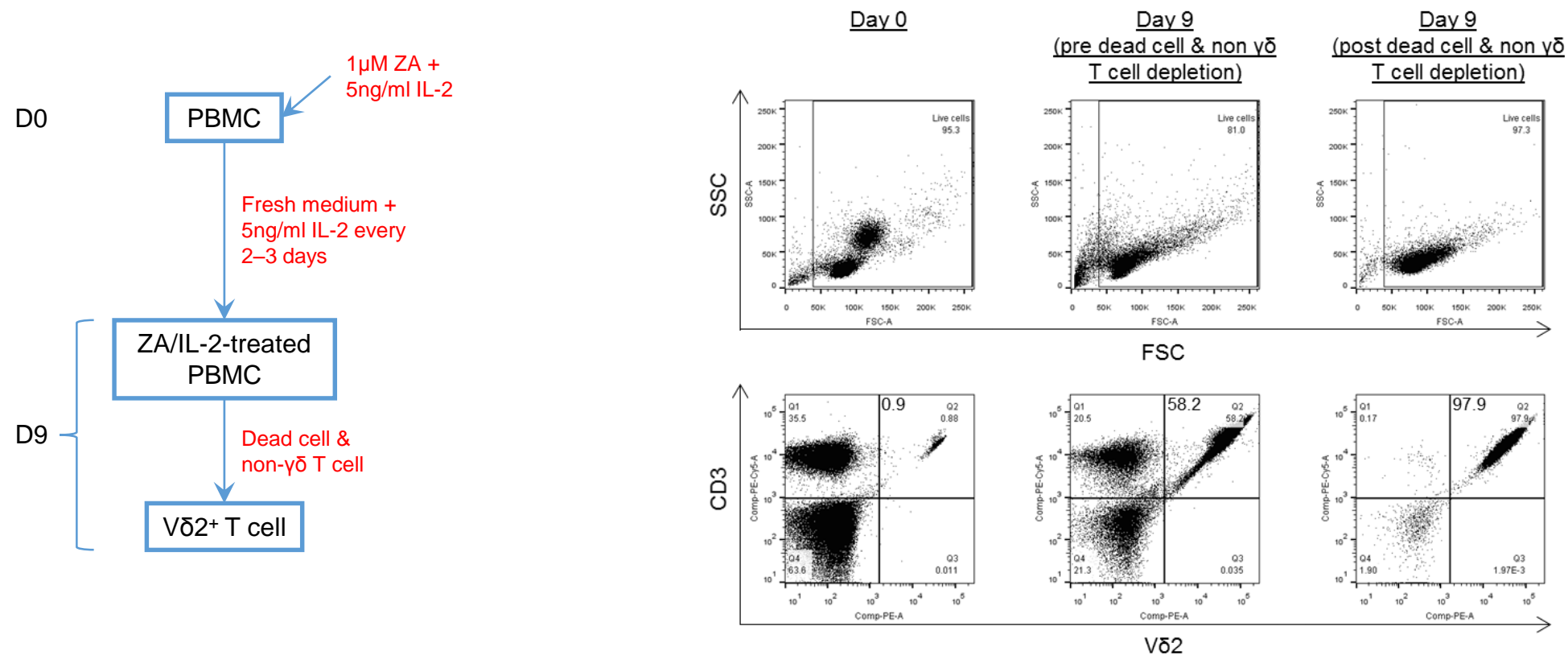




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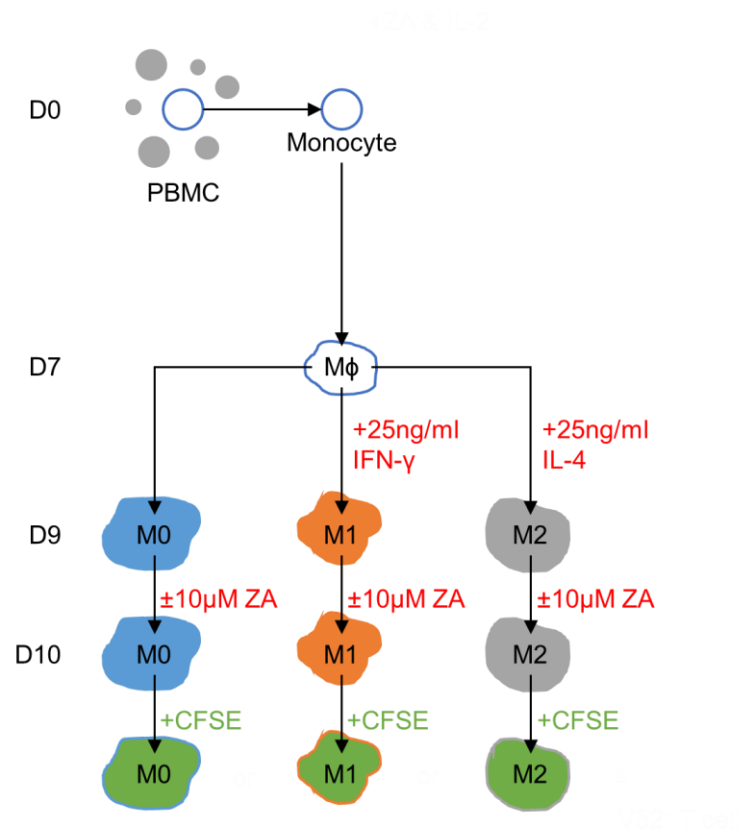


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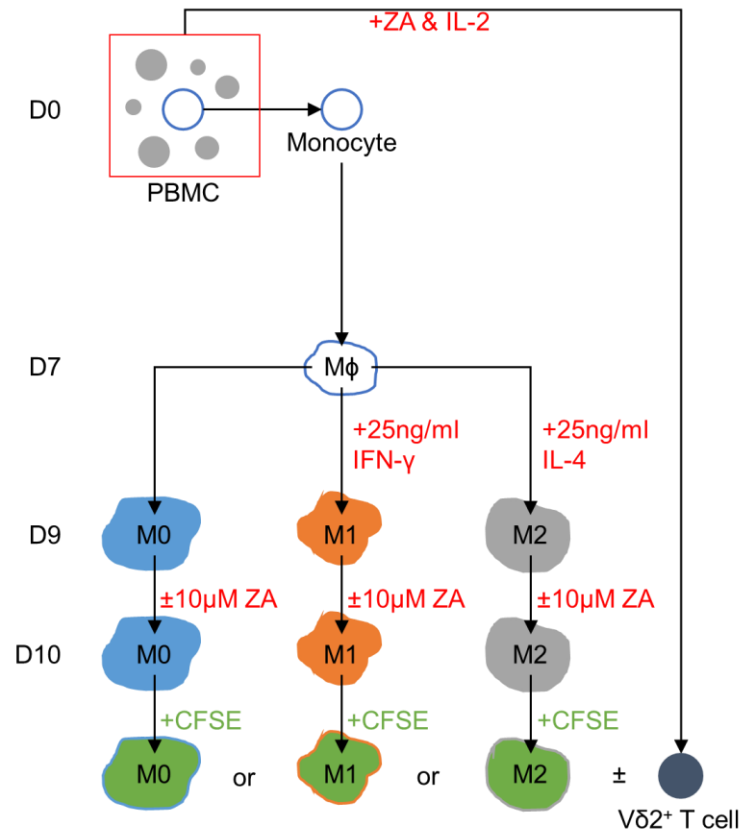


The diagram illustrates the experimental timeline for macrophage differentiation and treatment. At Day 0 (D0), PBMCs (Peripheral Blood Mononuclear Cells) are differentiated into Monocytes. At Day 7 (D7), the Monocytes are further differentiated into three macrophage populations: M0, M1, and M2. The differentiation is influenced by the addition of 25 ng/ml of IFN- $\gamma$  for M1 and 25 ng/ml of IL-4 for M2. At Day 9 (D9), all three populations (M0, M1, and M2) are treated with  $\pm 10 \mu\text{M}$  of ZA (Zinc Acetate). The diagram shows the progression from D0 to D10, with the final state of the macrophages at D10.

# V $\delta$ 2<sup>+</sup> T cell cytotoxicity

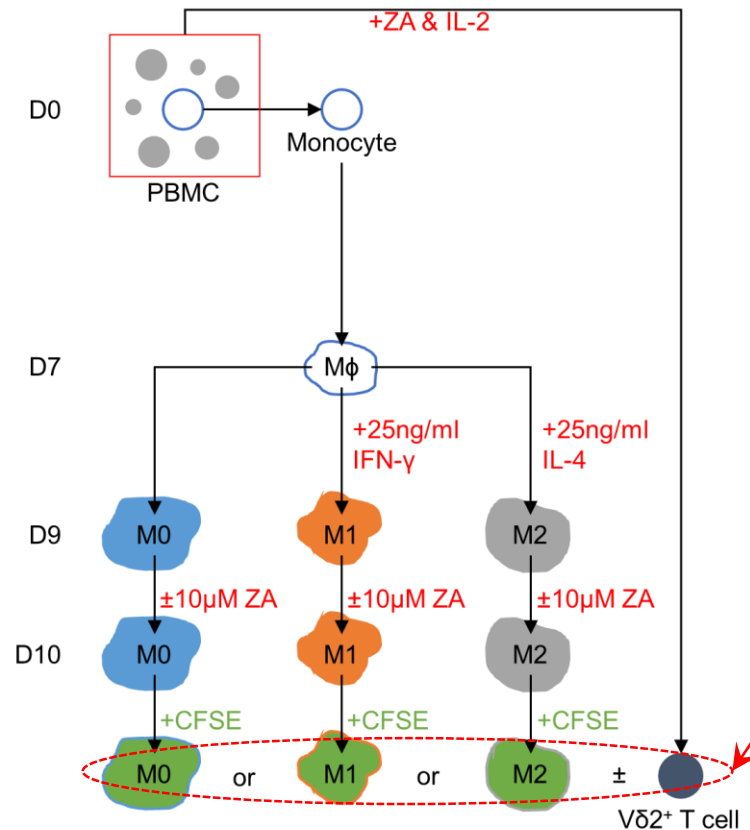


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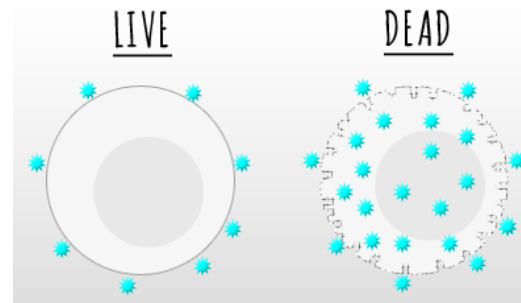


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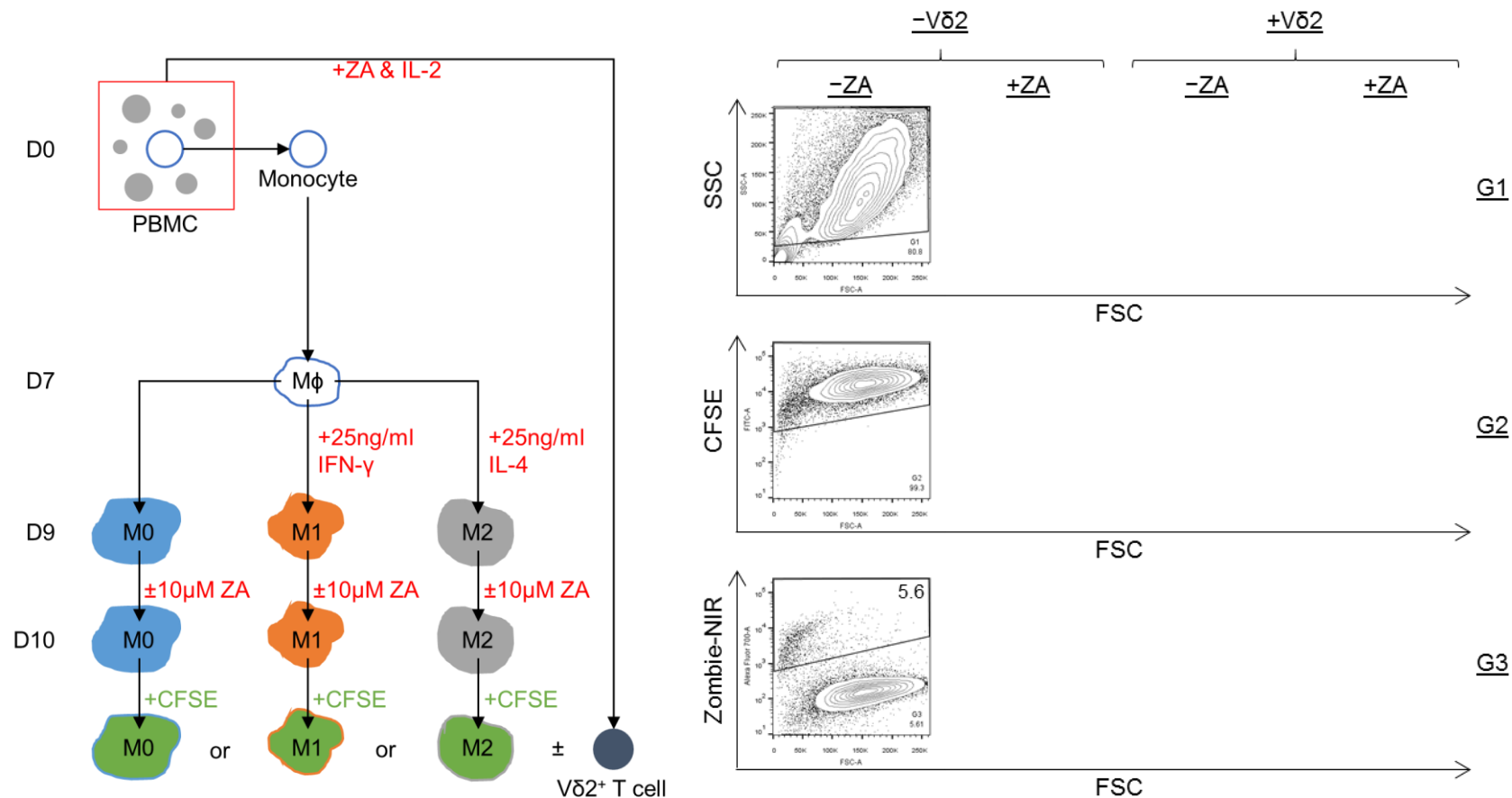


Flow cytometry to measure Zombie-NIR expression by CFSE<sup>+</sup> cells

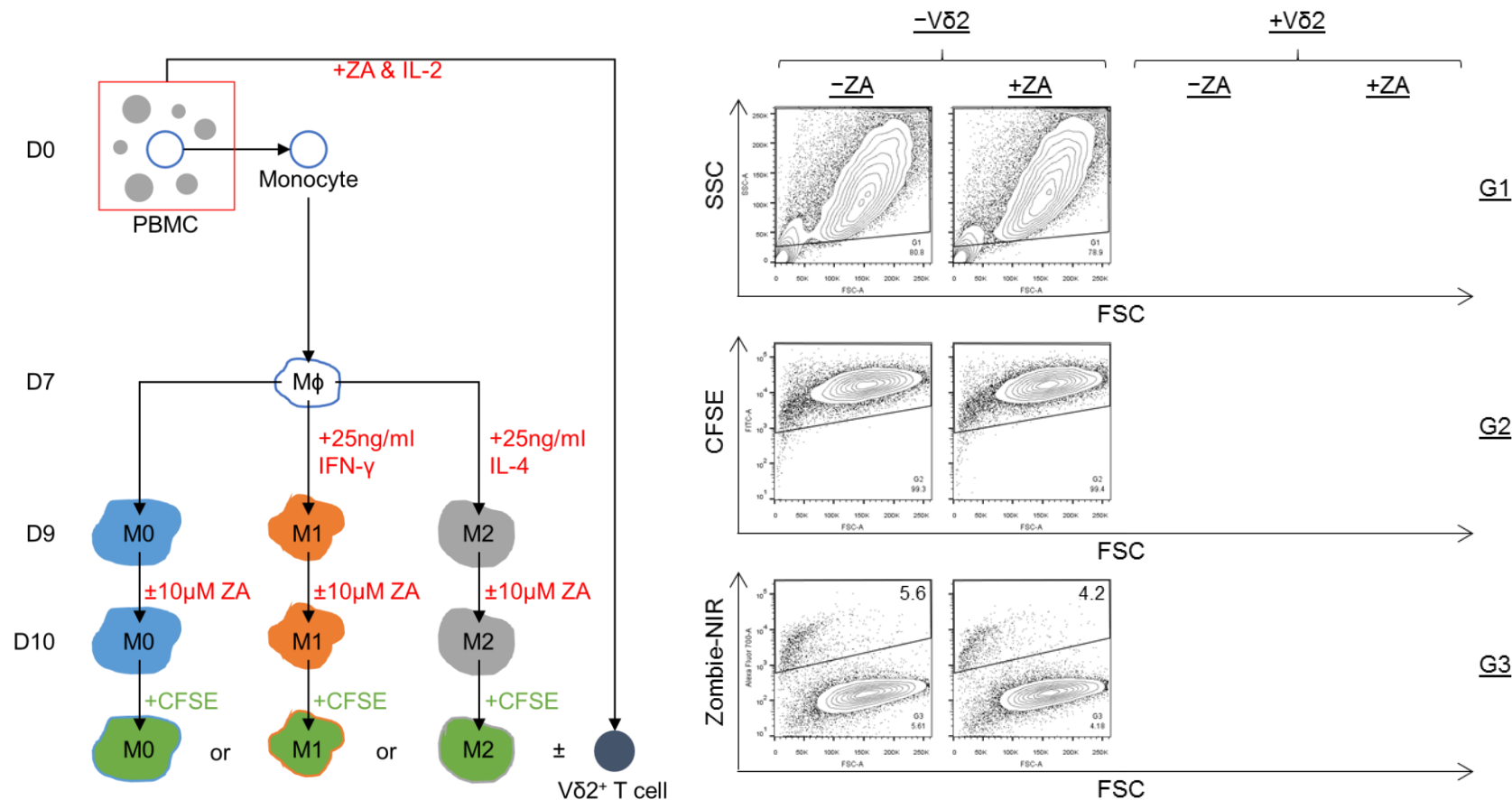
Zombie-NIR live/dead cell discrimination dye



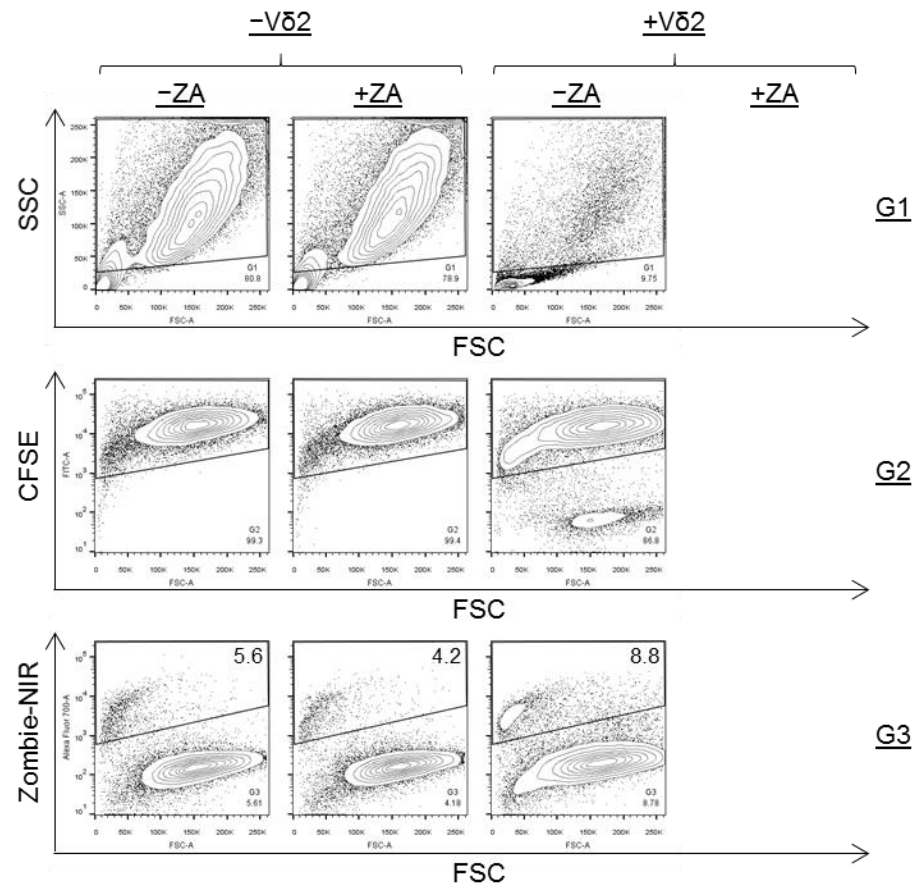
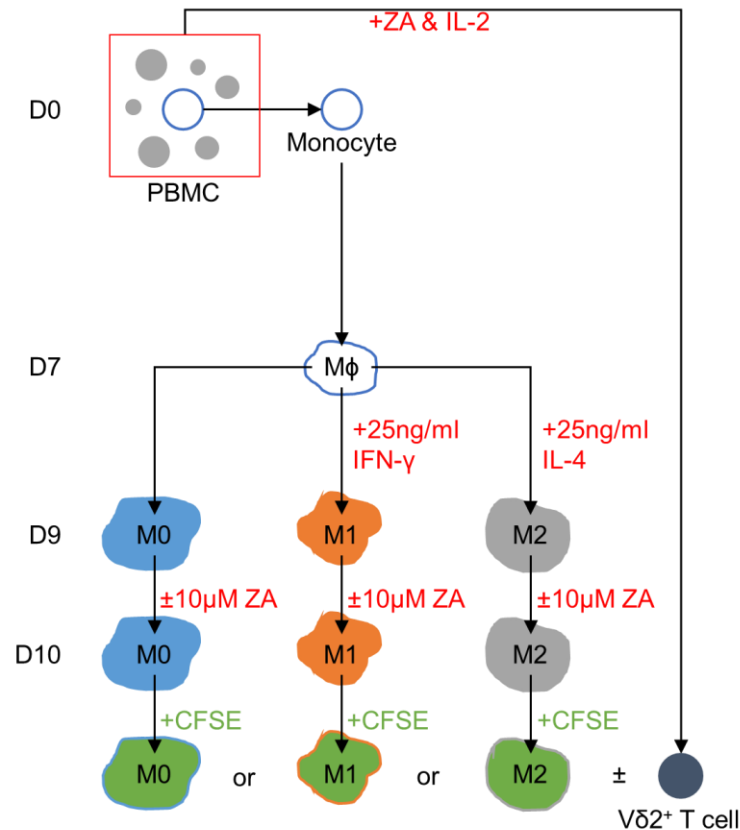
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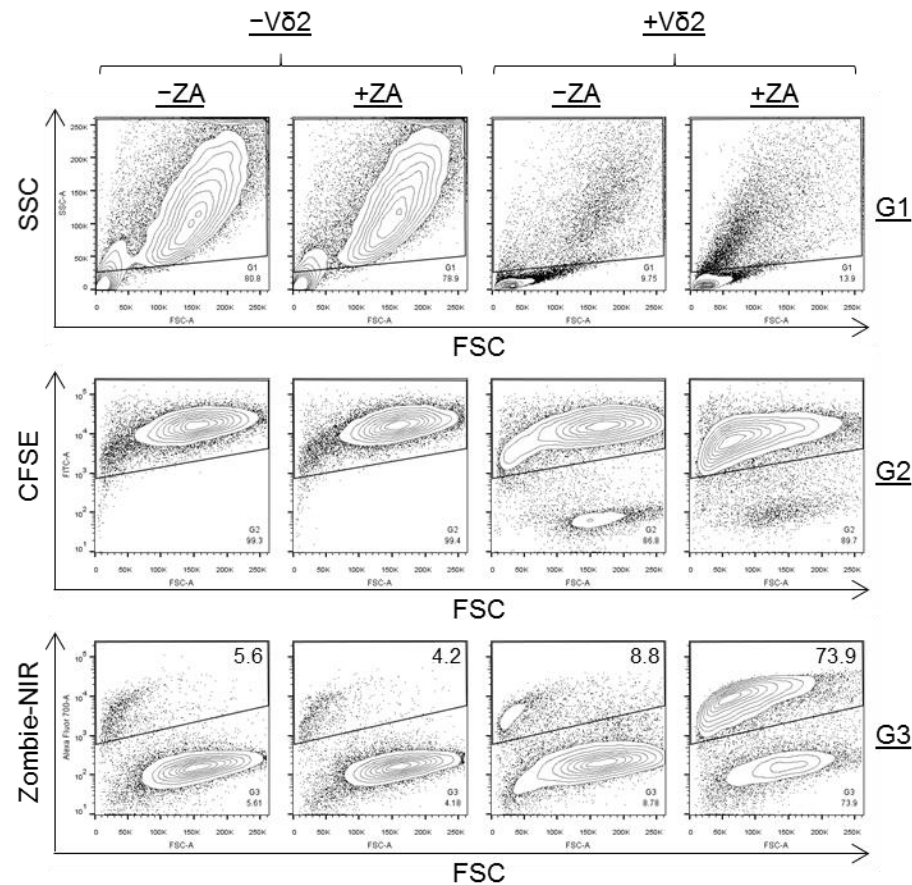
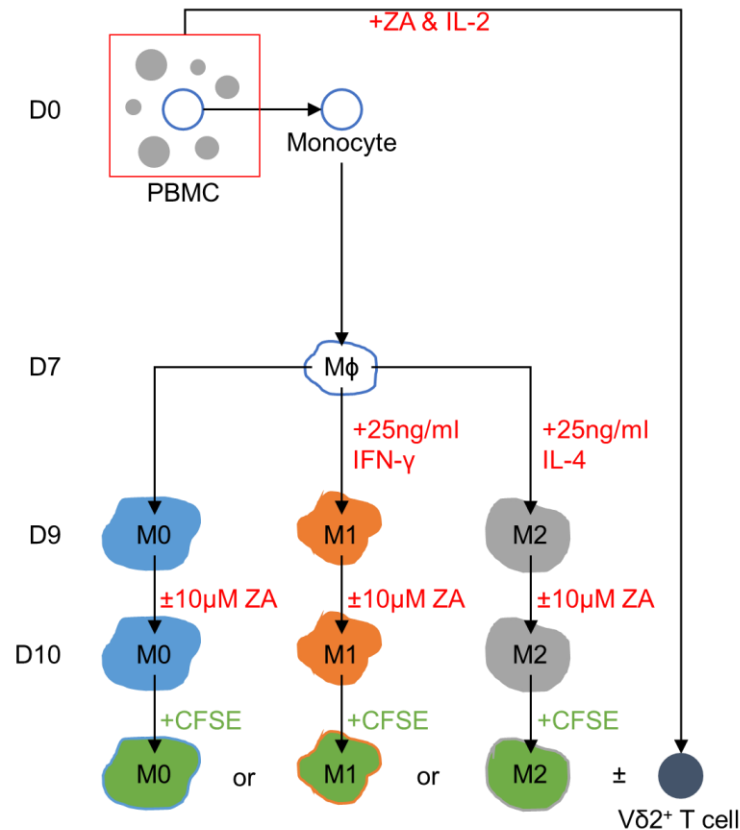
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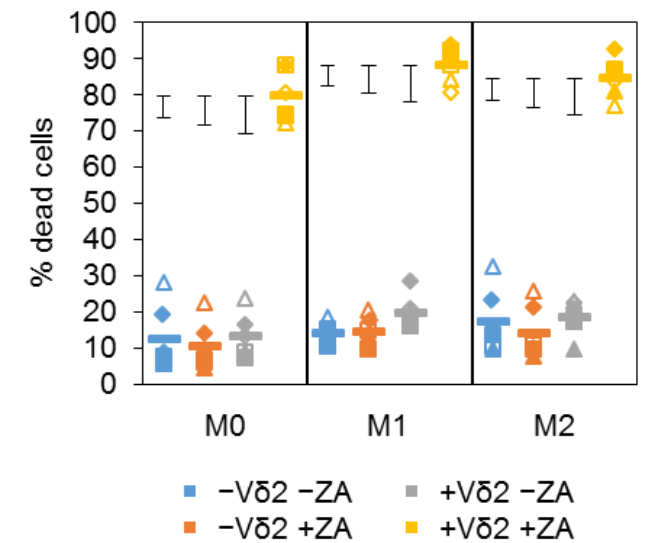
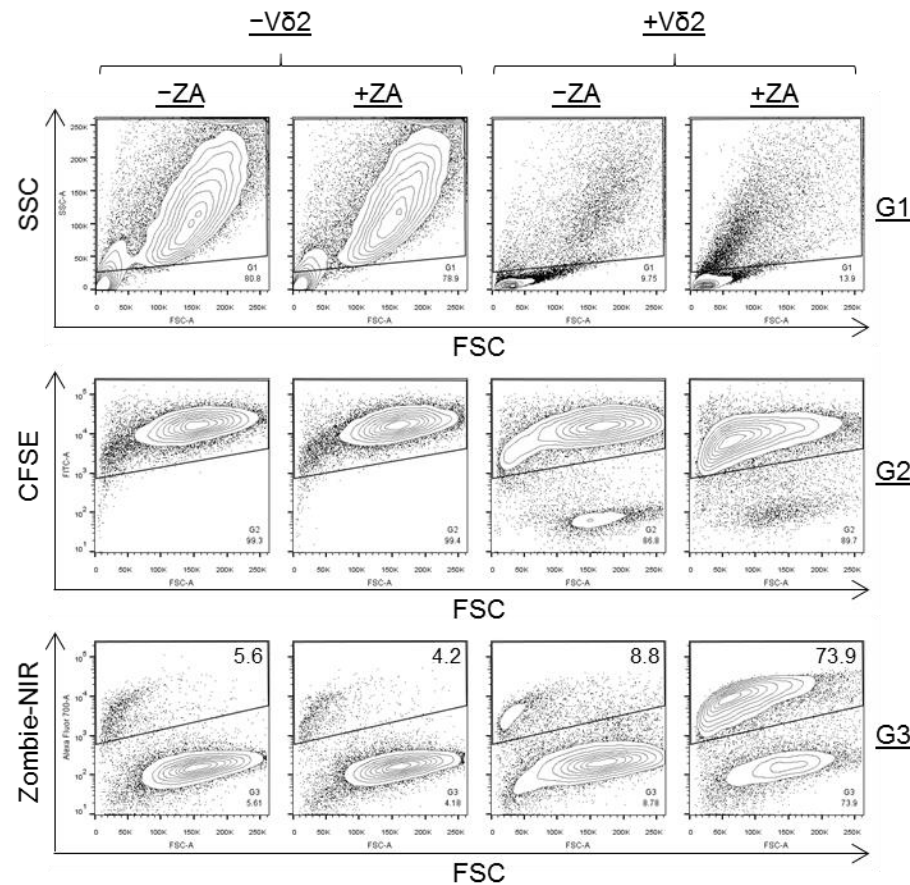
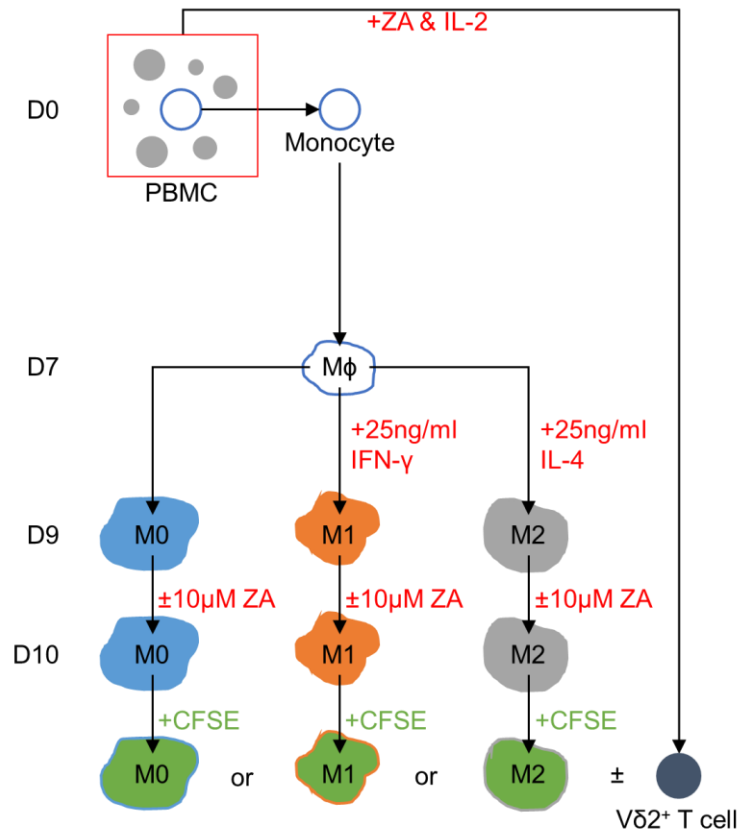


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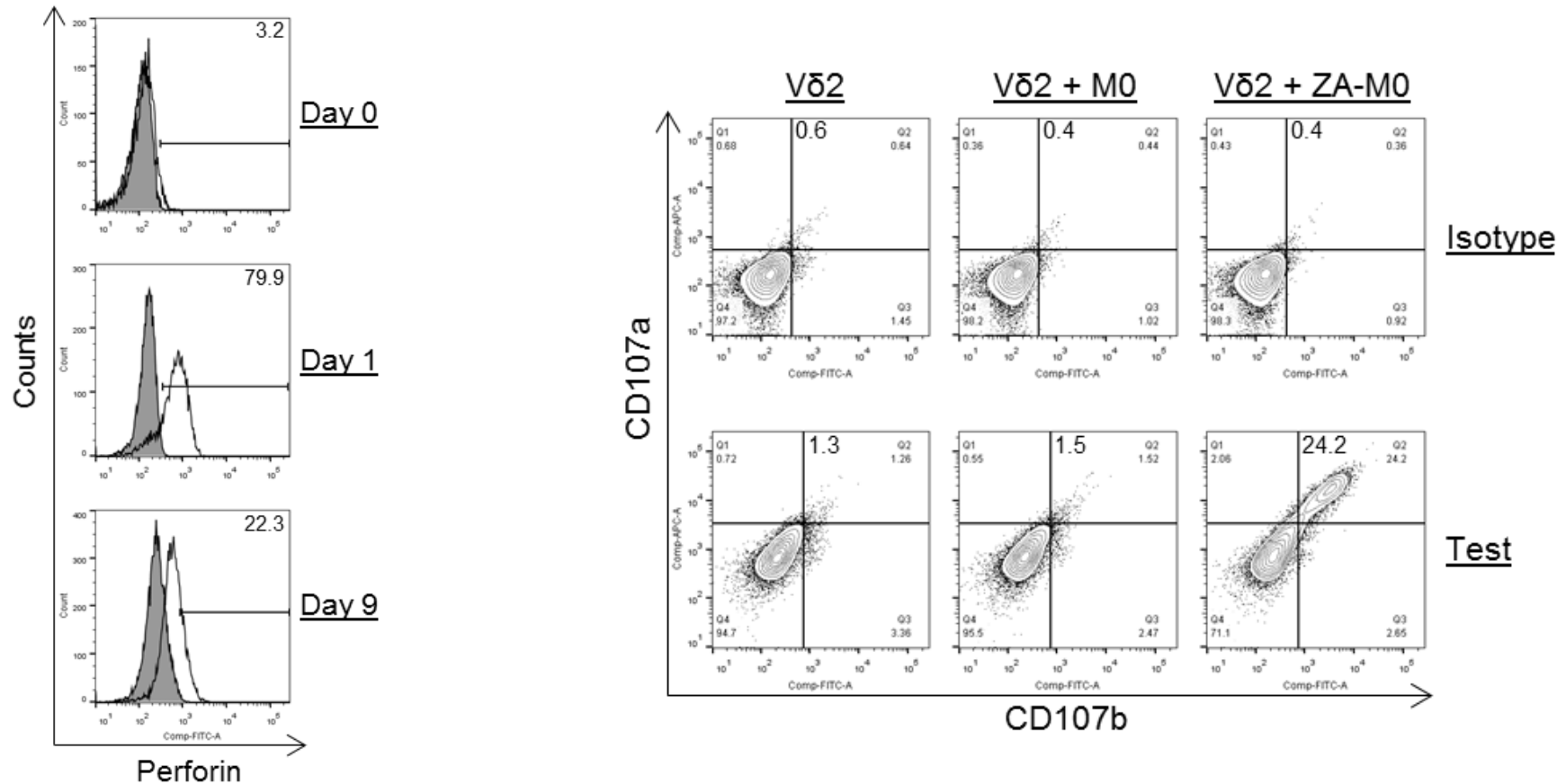




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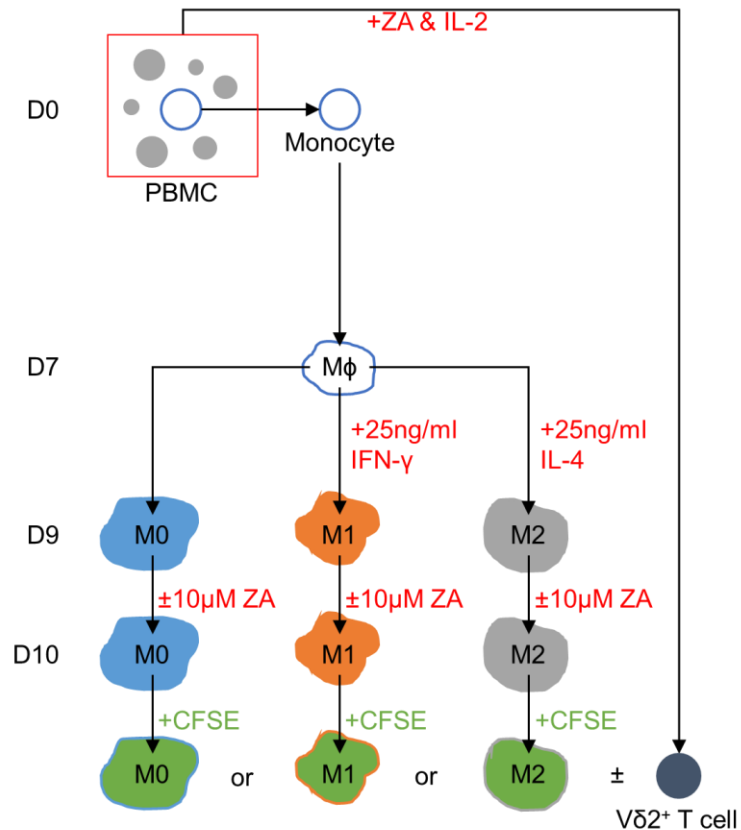


# Perforin & degranulation

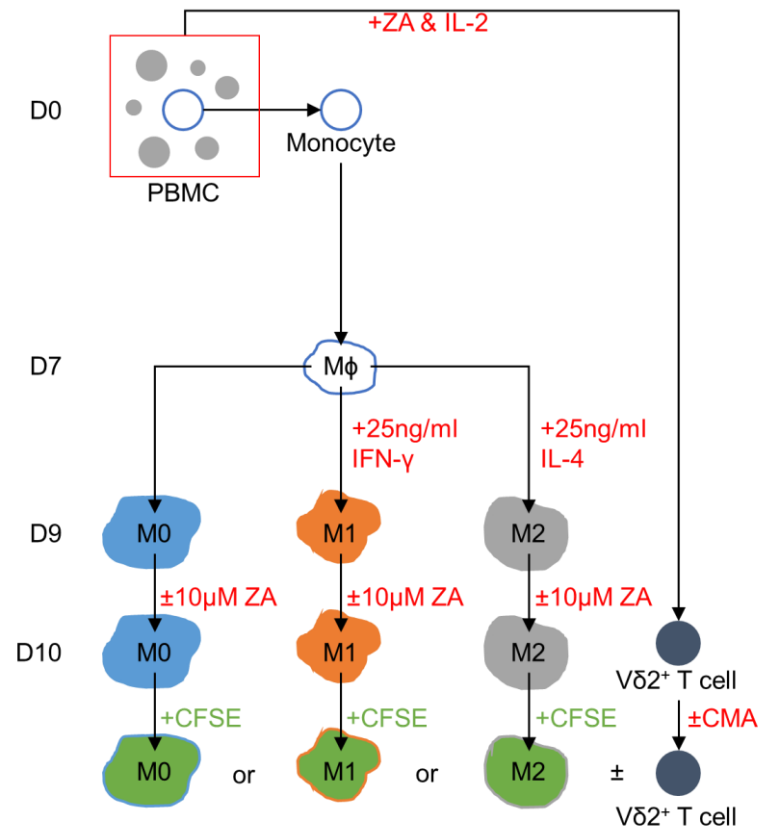


Consistent for three donors  
Same effect for M1 & M2 Mφs

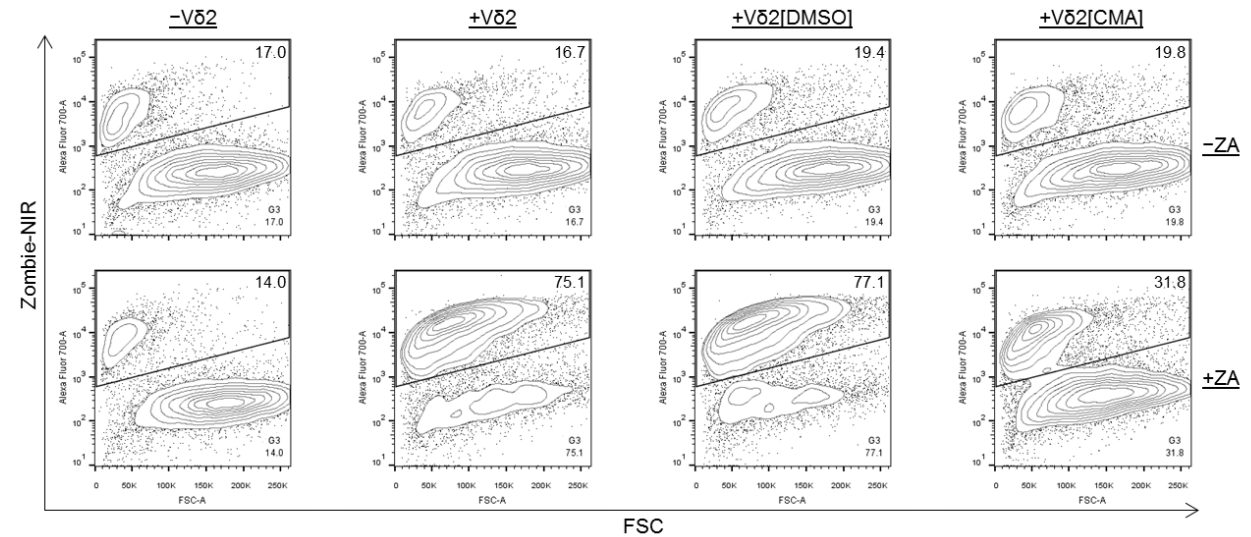
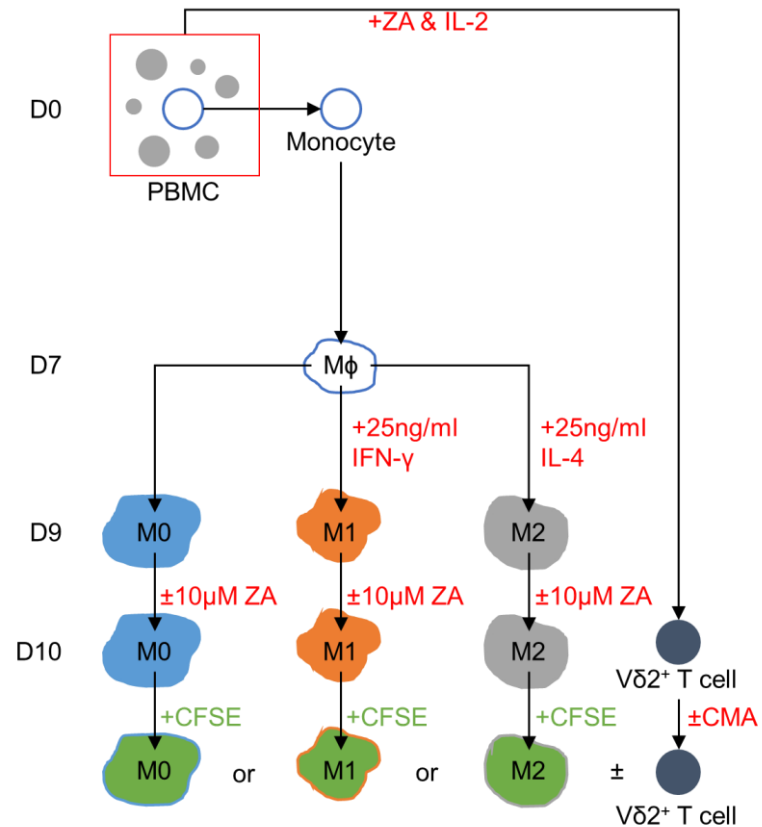
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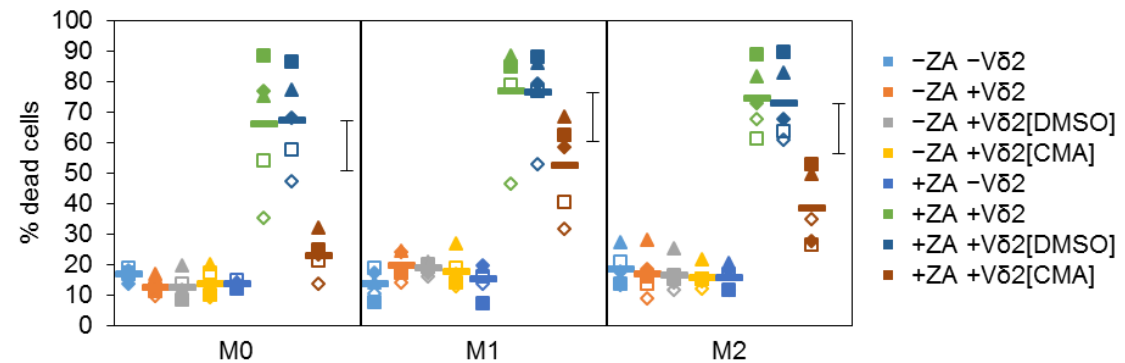
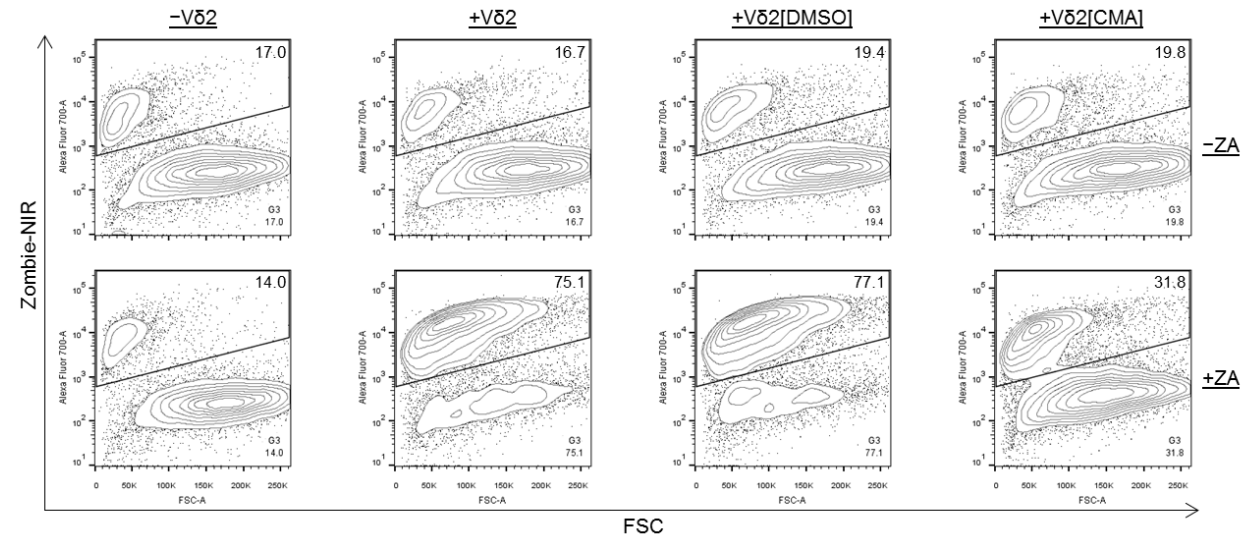
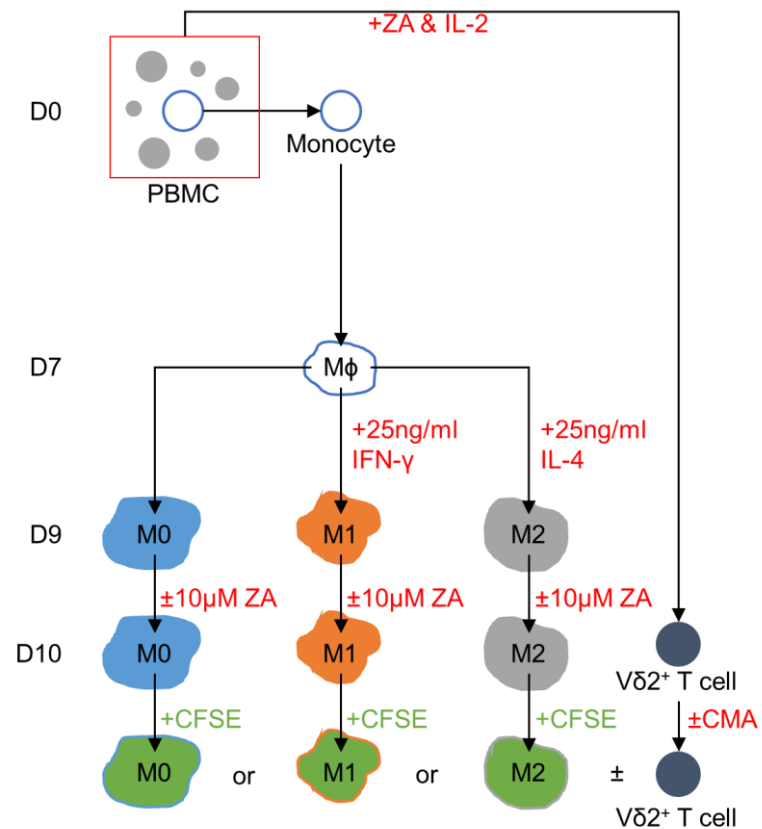


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# Summary

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## ☐ **ZA did not affect M $\phi$ phenotype or viability**

- ☐ Apoptosis & repolarisation reported previously
- ☐ Short culture & no markers of early apoptosis

## ☐ **ZA rendered M $\phi$ s susceptible to V $\delta$ 2<sup>+</sup> T cell cytotoxicity**

- ☐ Killing at 10 $\mu$ M but not 1 $\mu$ M suggests that M $\phi$ s associated with calcified tissues where ZA is known to accumulate are most likely to be susceptible to this effect
- ☐ No difference in susceptibility between M1 & M2 highlights the need to understand the pro- vs. anti-tumour effects of M $\phi$ s in patients treated with ZA

## ☐ **Cytotoxicity was perforin dependent**

- ☐ Perforin expression, degranulation & sensitivity to concanamycin A
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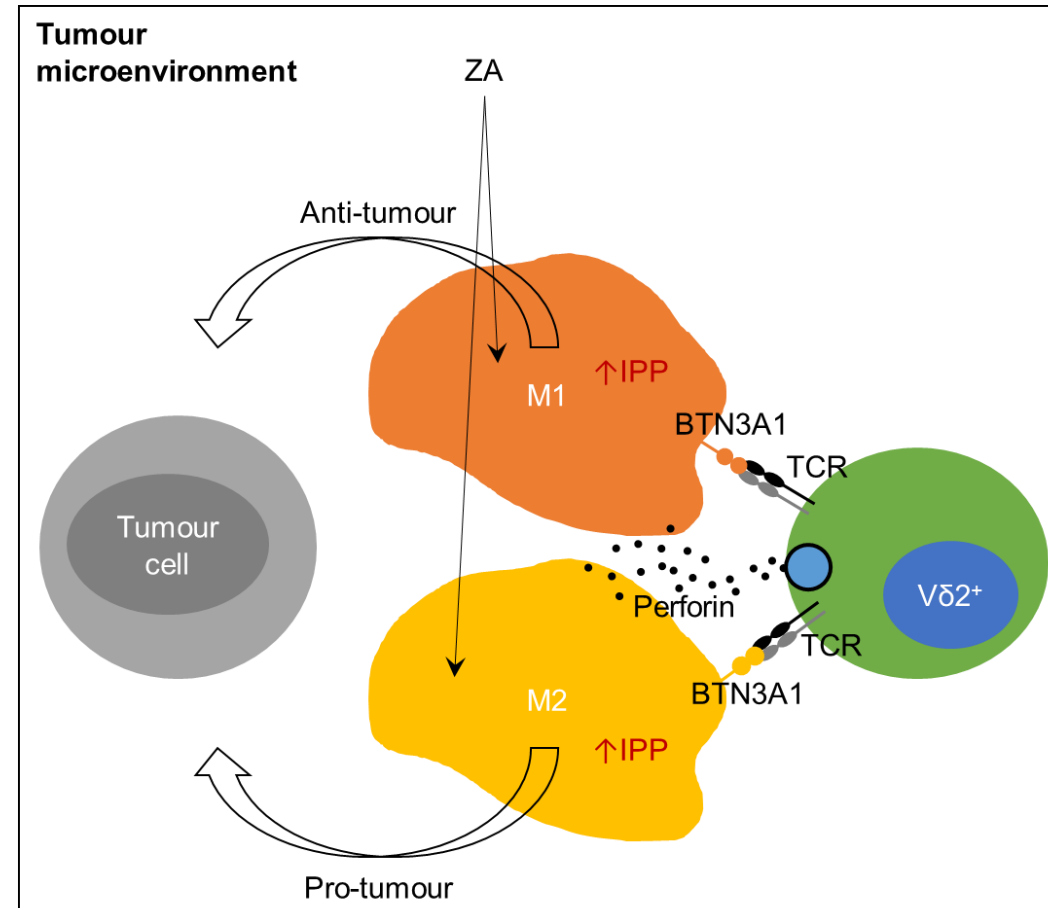
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# Take home message

**ZA can kill M1 & M2 M $\phi$ s indirectly by rendering them susceptible to perforin-mediated cytotoxicity by V $\delta$ 2<sup>+</sup>  $\gamma\delta$  T cells**



# Acknowledgments

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## ☐ Research team

Dr Mark Bodman-Smith  
Dr John Copier  
Professor Angus Dalglish

## ☐ Advice on statistical analyses

Dr David Lovell

## ☐ Funding



[icvi.org.uk](http://icvi.org.uk)