Using spectral cytometry to investigate novel pre-conditioning regimens in a congenic adoptive immunotherapy mouse model

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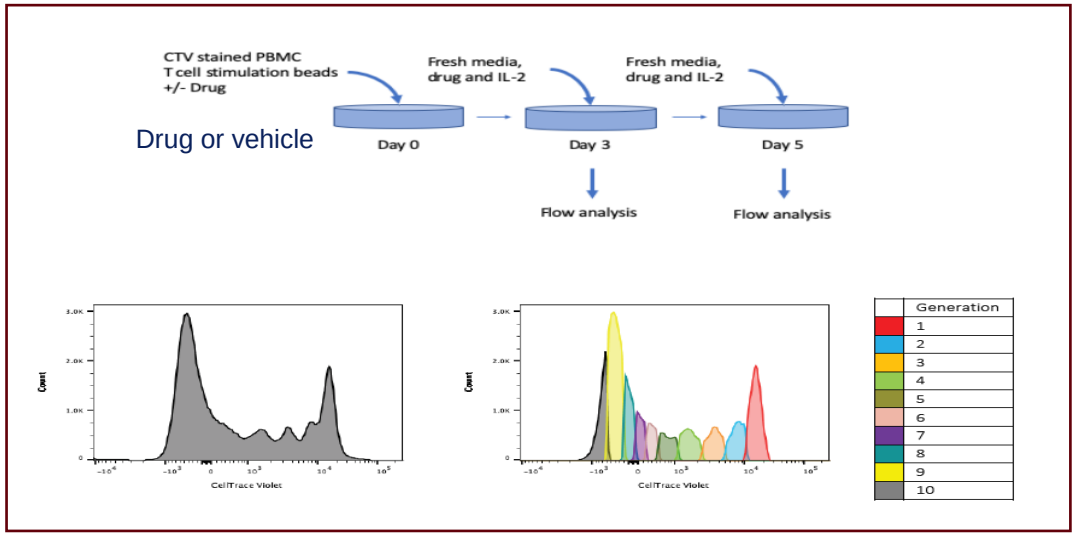
ACRF Translational Research Laboratory

- Interested in human and mouse immune cell function
- Effects of novel immunotherapies on immune system in patients with blood cancer
- Effects of repurposing drugs to ablate the immune system prior to stem cell transplant



Combine cell trace violet (CTV)

- Proliferation
- Killing
- Intracellular cytokine staining
- Checkpoint markers
- In vivo mouse models of adoptive immunotherapy

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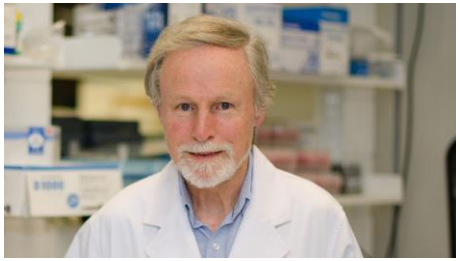
Cell proliferation analysis



- Pioneered by Prof. Chris Parish in 1990

- CFSE (1990)
- CellTrace Violet (2012)

Journal of Immunological Methods, 133 (1990) 87–97
Elsevier

JIM 05698

New fluorescent dyes for lymphocyte migration studies

Analysis by flow cytometry and fluorescence microscopy

Susan A. Weston and Christopher R. Parish
Division of Cell Biology, John Curtin School of Medical Research, Australian National University, Canberra, A.C.T. 2601, Australia

(Received 20 April 1990, revised received 7 June 1990, accepted 11 June 1990)

ELSEVIER

Journal of Immunological Methods 171 (1994) 131–137

JOURNAL OF IMMUNOLOGICAL METHODS

Determination of lymphocyte division by flow cytometry

A. Bruce Lyons *, Christopher R. Parish
Division of Cell Biology, John Curtin School of Medical Research, Australian National University, Canberra, A.C.T. 2601, Australia

(Received 4 January 1994; accepted 22 February 1994)

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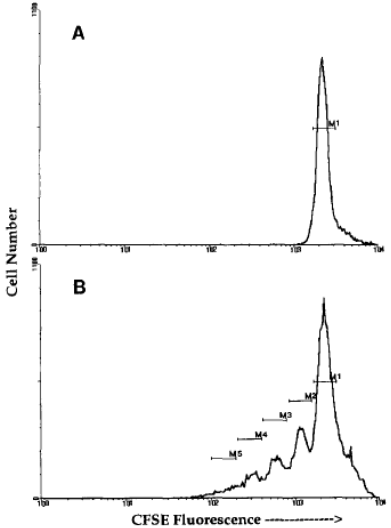
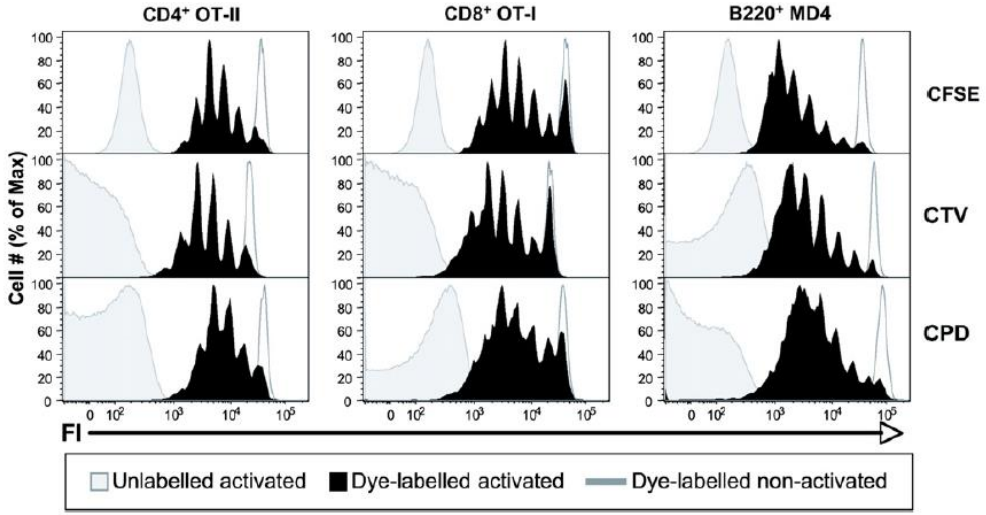
Cell proliferation analysis – CFSE & Cell Trace Violet (CTV)





Lyons and Parish 1994, Quah and Parish 2012

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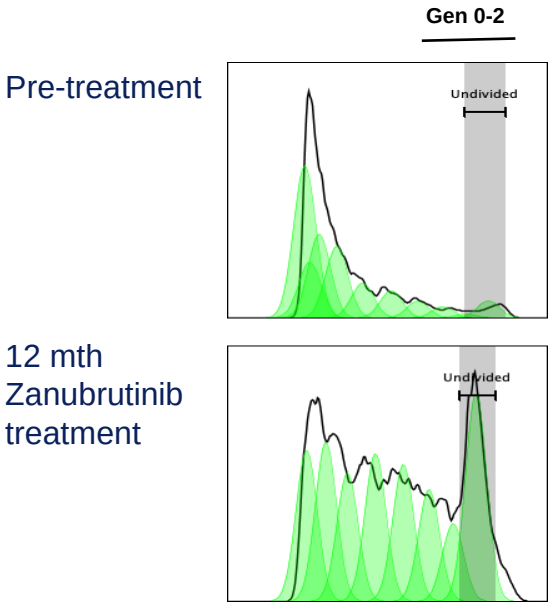
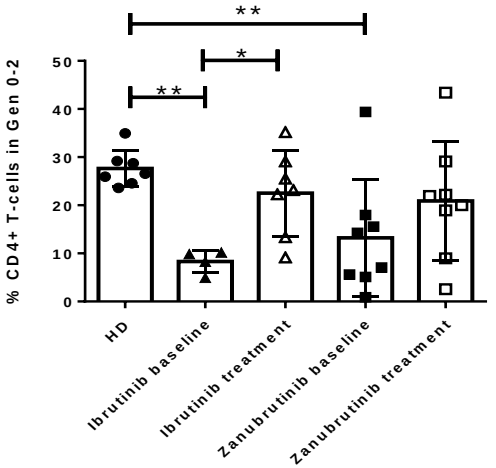
BTKi treatment inhibits T cell hyperproliferation in Chronic Lymphocytic Leukaemia (CLL) patient blood samples





Davis, J. Trans. Med. 2021

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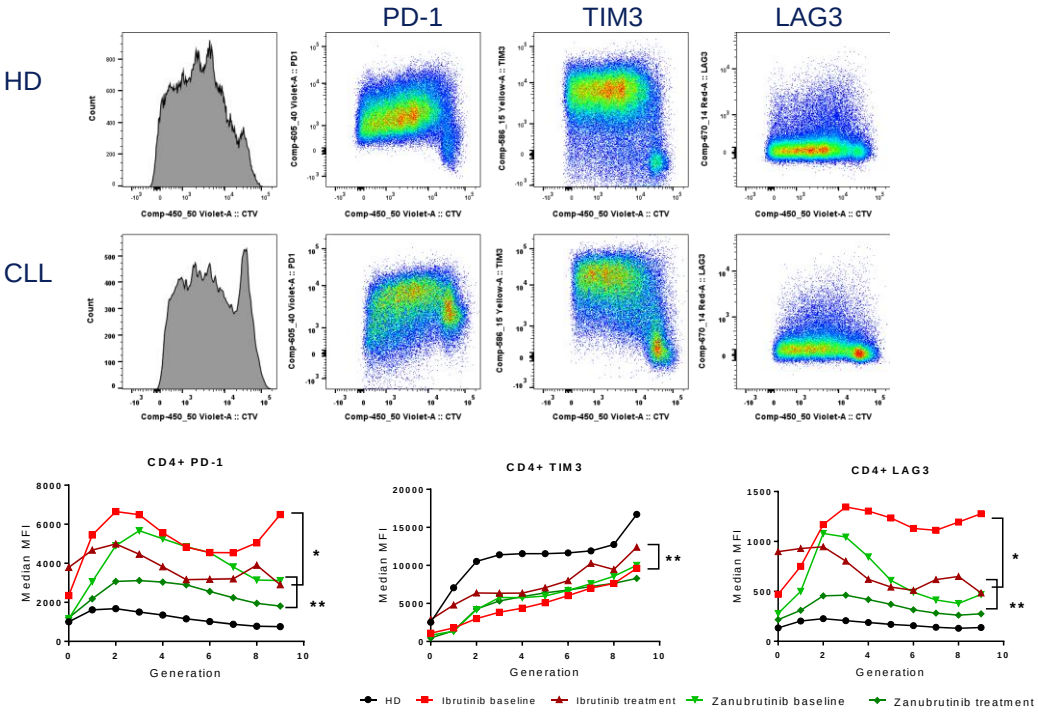
BTKi treatment reduces CLL T cell checkpoint marker expression



Davis, J Trans. Med. 2021

Poster #4

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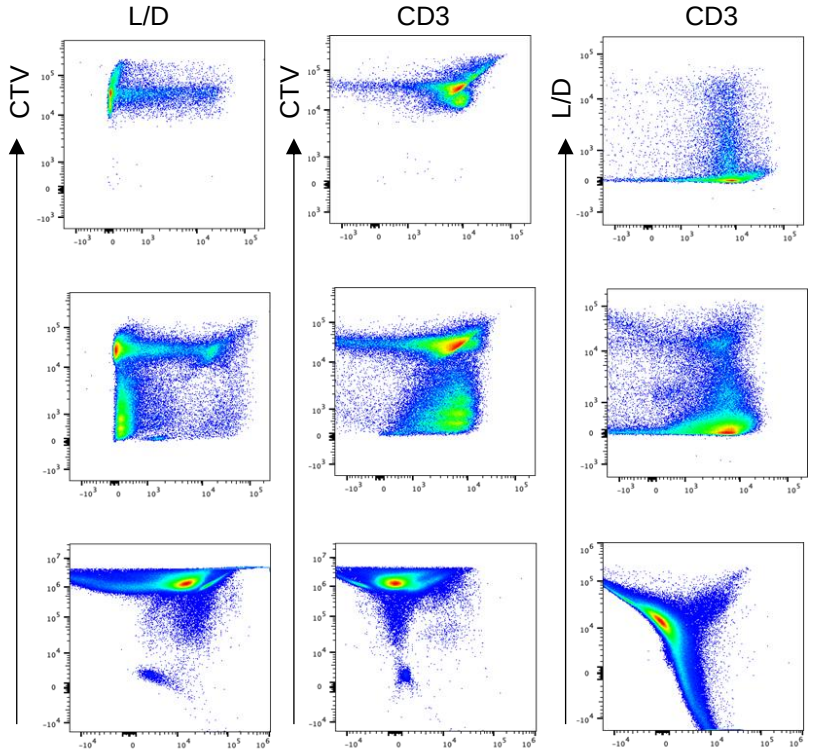
CTV technical issues

- Cell loss after labelling with CTV
- Transition to spectral cytometry
  - unmixing errors



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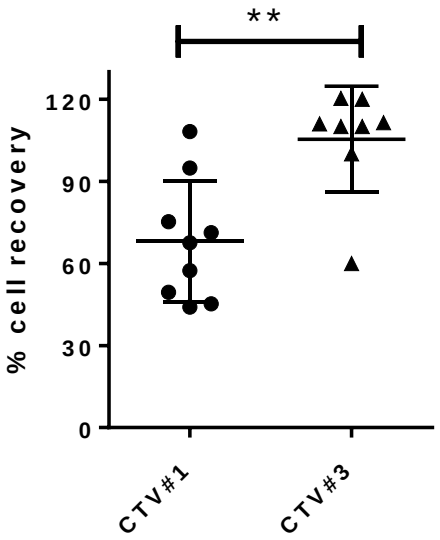
Comparing CTV labelling methods

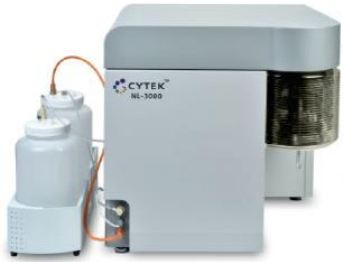
Method	CTV dilution, final concentration	Staining buffer and cell concentration	Temperature and incubation time
CTV#1	1:2000, 2.5 μM	PBS, $1x10^6$/ml	37°C, 20 min
CTV#2	1:500, 10 μM	R10, $1x10^7$/ml	RT, 5 min
CTV#3	1:5000, 1 μM	PBS + 2% FCS, $1x10^7$/ml	37°C, 20 min



CTV#1 – manufacturer's method
CTV#2 – adapted from Quah and Parish 2012
CTV#3 – Davis et al, Cytometry Part A 2024

Comparing CTV labelling methods

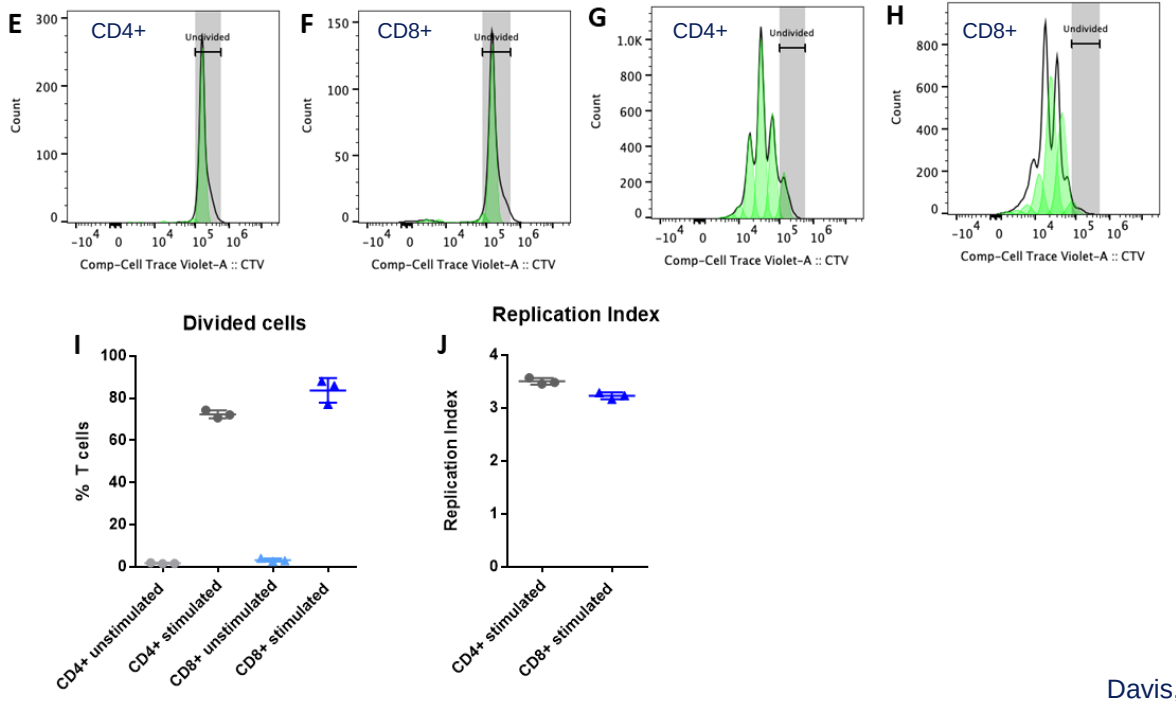
Cytek Aurora (5L)



Doherty Institute,
Melbourne Cytometry Platform

Proliferation analysis performed using FlowJo software

CTV#3 – in vitro stimulation of mouse T cells



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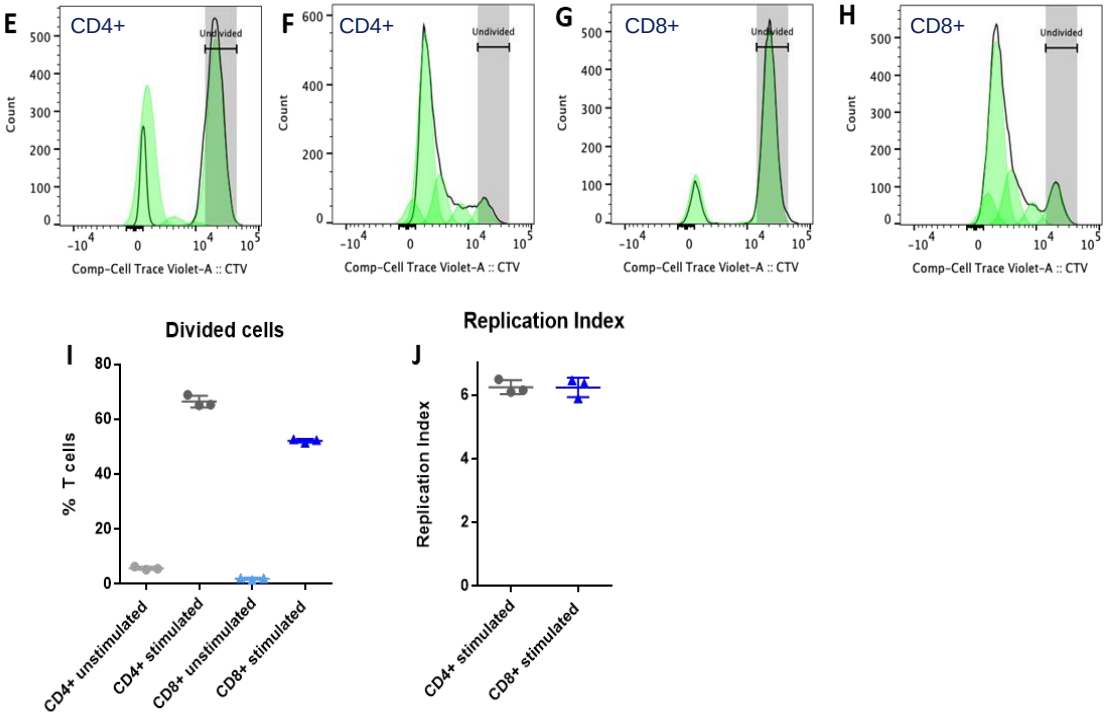
CTV#3 – in vitro stimulation of human T cells



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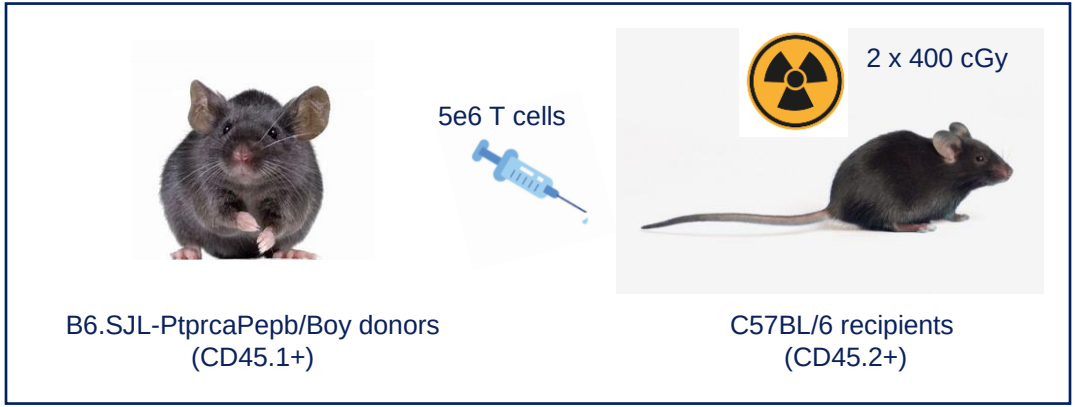
CTV#3 – in vivo congenic mouse model



Days 7 & 14, perform analysis on splenic and LN T cells

Examine proliferation of donor T cells, migration to LN, memory phenotype

CTV and congenic mouse immune panel

Laser	Specificity	Fluorochrome	Optimised dilution
UV 2	CD8	BUV395	1/400
UV 6	Viability	Live/Dead Blue	1/1000
UV 7	CD45	BUV496	1/1600
UV 9	CD4	BUV563	1/800
UV14	CD69	BUV737	1/400
UV 16	NK1.1	BUV805	1/100
V 1	Ly6G	BV421	1/400
V3	CTV	CTV	1/5000
V 8	CD19	BV570	1/100
V 10	CD11b	BV605	1/1000
V 11	CD3	BV650	1/100
V 13	CD44	BV711	1/200
R 2	CD49d	Alexa 647	1/100
R 4	Ly6C	A700	1/400
R7	CD45.2	APC-e780	1/200
B 2	CD45.1	FITC	1/400
YG 9	CD62L	PE-Cy7	1/400

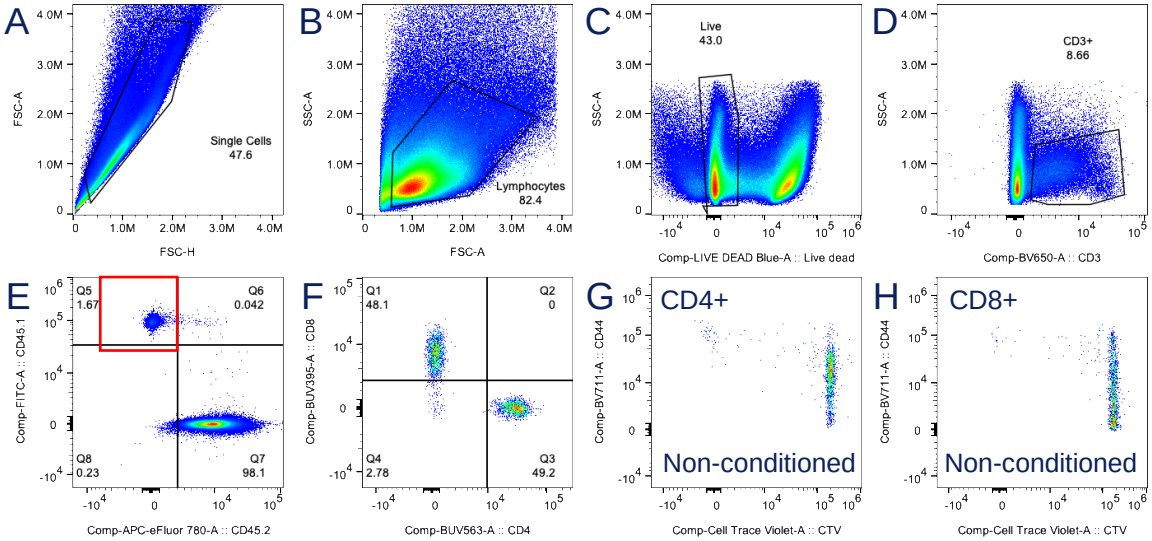
CD45.1+ vs CD45.2+

CTV donor cells

Memory phenotype

Recipient immunity

Identification of donor T cells 7 days after infusion



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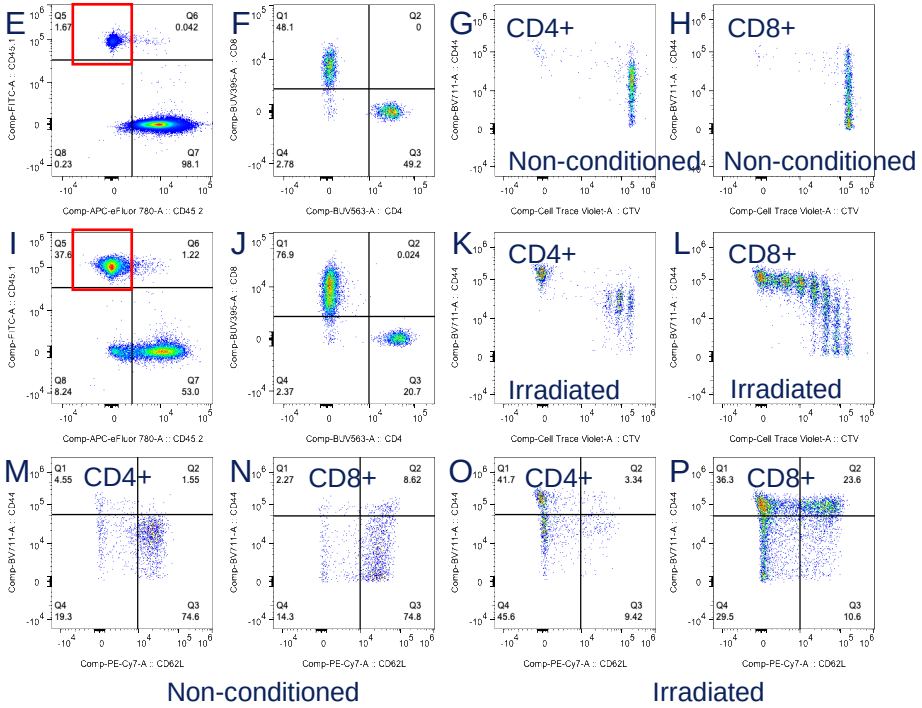
Donor cells proliferate in irradiated recipients



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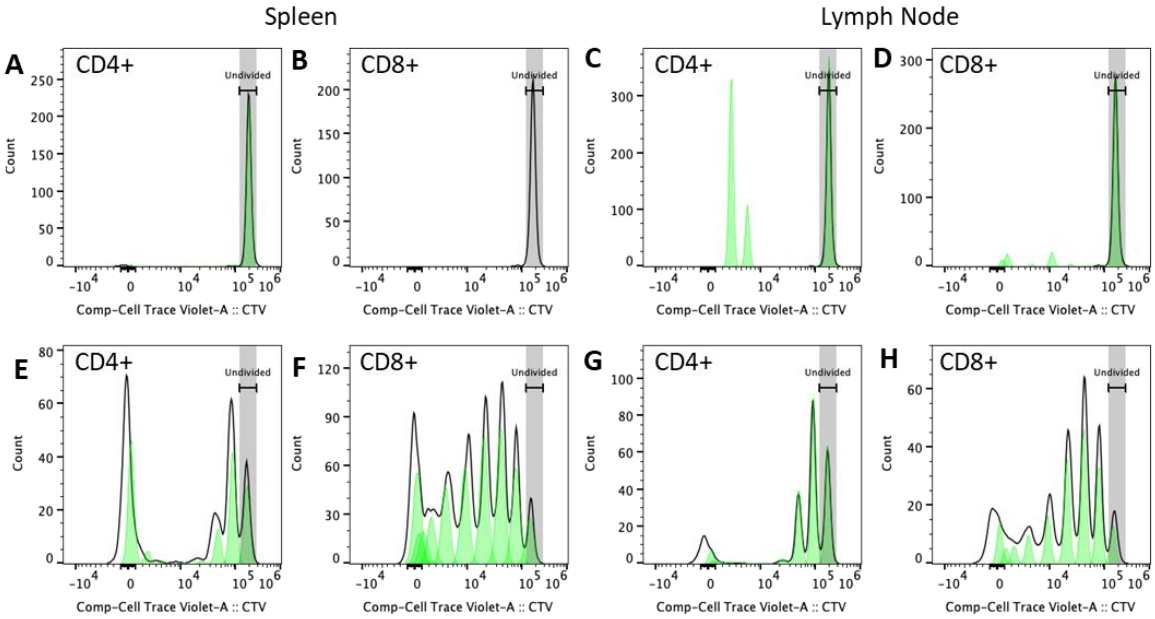
Differential proliferation between CD4+ and CD8+ T cells



Davis, Cytometry Part A, 2024

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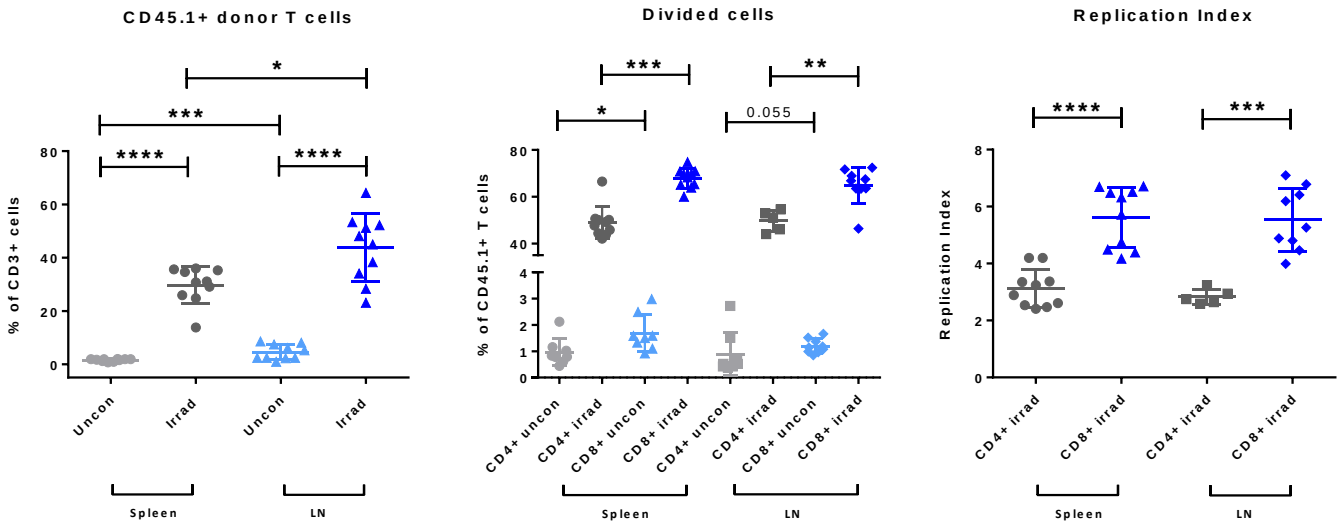
CD8+ T cells divided more rapidly than CD4+ T cells



Davis, Cytometry Part A, 2024

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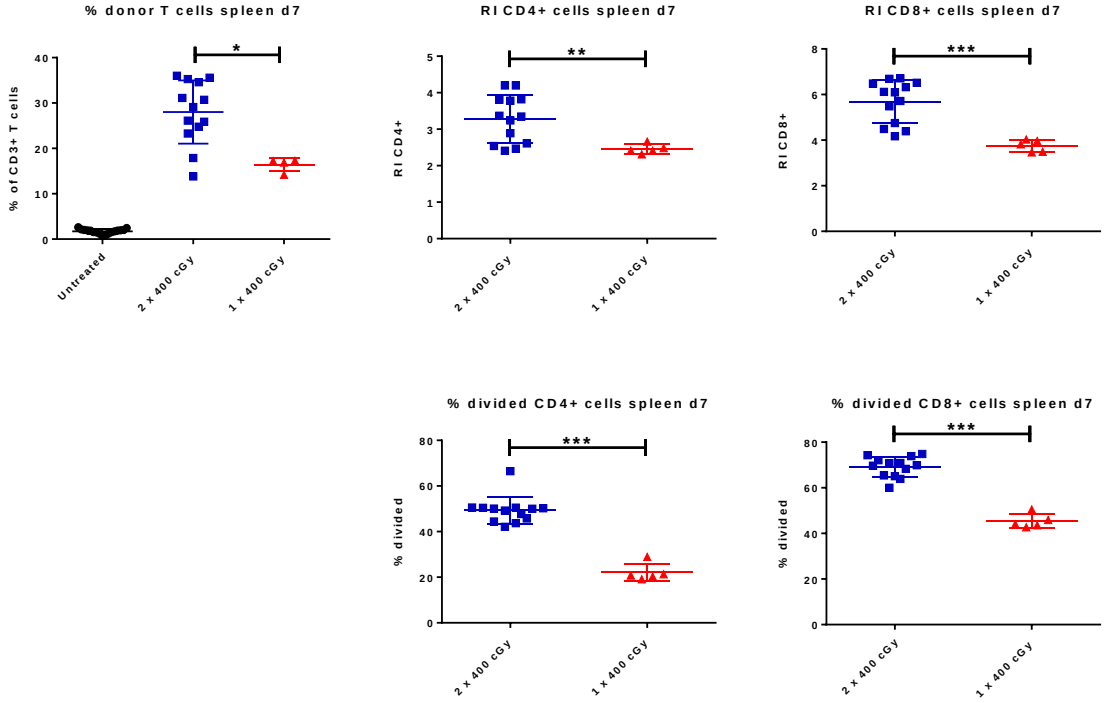
Irradiation dose affects donor cell proliferation



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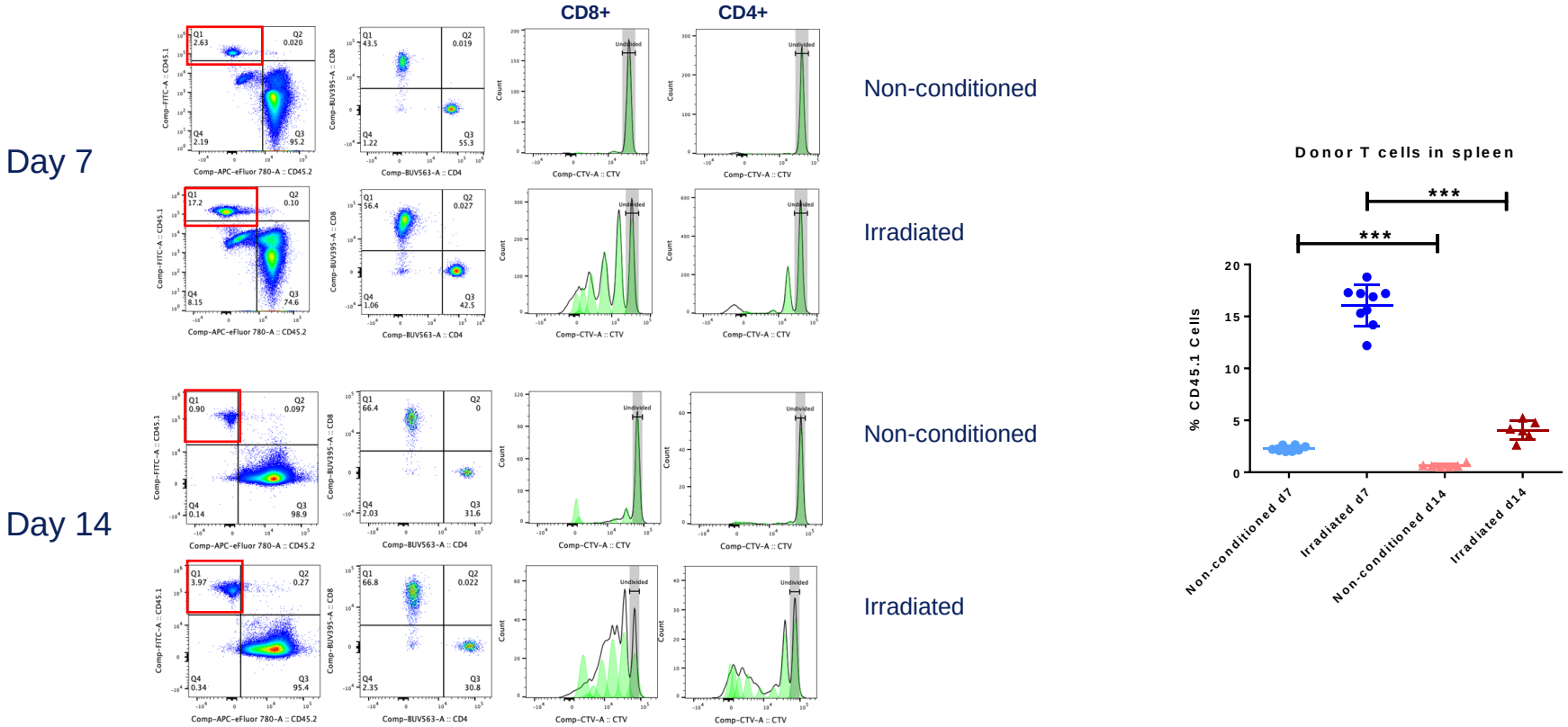
Time post-infusion affects donor cell proliferation and recovery



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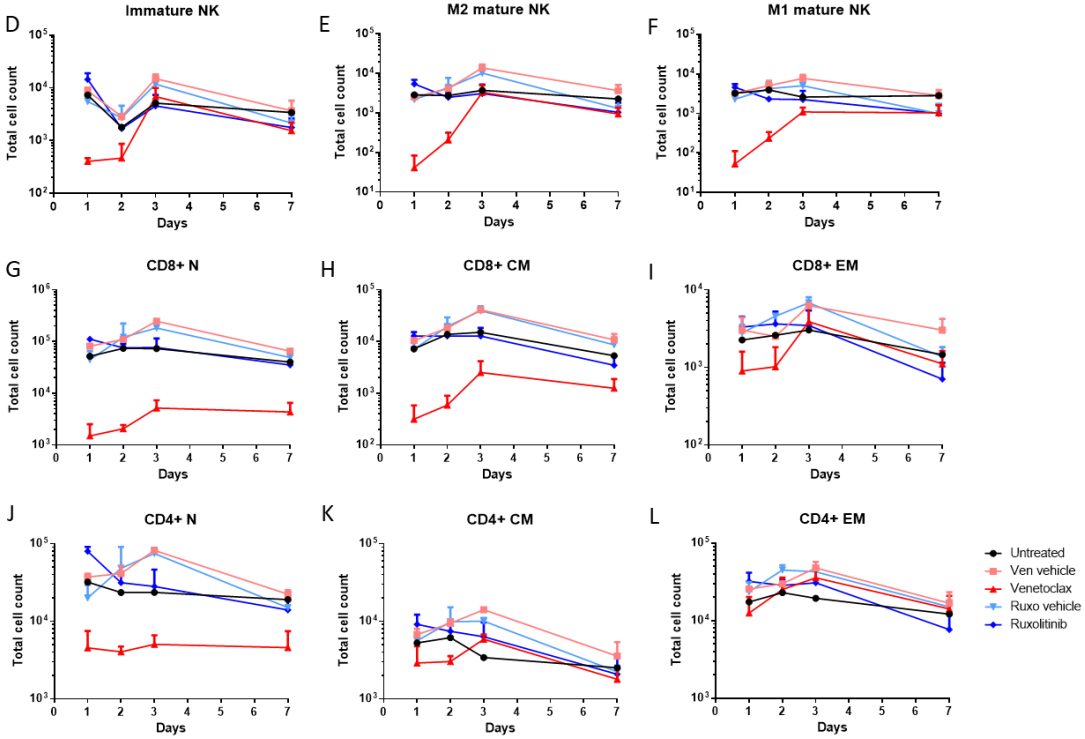
Role of pre-conditioning recipient immune ablation

- Irradiation
- Venetoclax
- Combinations

Potential therapies – immune ablation in allogeneic SCT mouse models and Phase I clinical trial (VICTORY)

Adoptive immunotherapy of donor T cells (CAR T)

Venetoclax transiently depletes recipient NK and T cells



Davis, Frontiers Immunol., 2021

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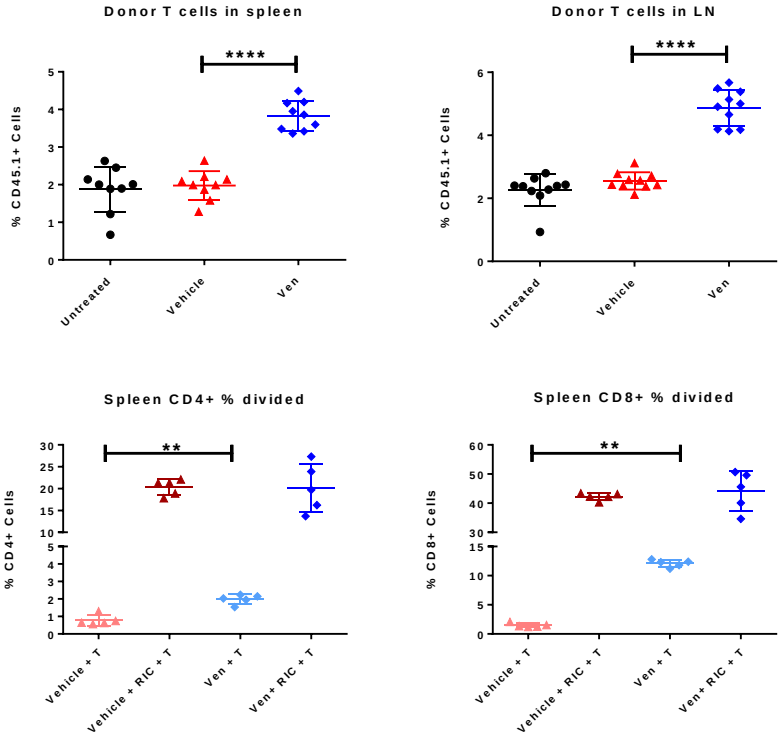
Venetoclax treatment increases donor T cell expansion



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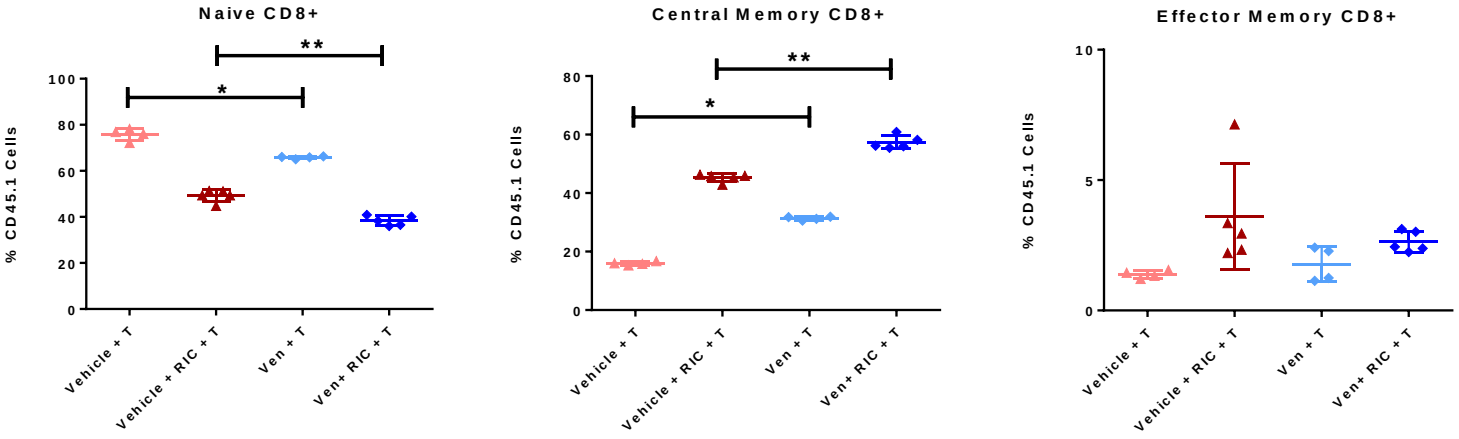
Venetoclax treatment increases central memory phenotype



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Conclusions

Modified CTV labelling method (2% FCS, 1 μM CTV, 10^7 cells/ml, 20 min @ 37°C)

- Improves cell recovery after labelling
- Avoids spill-over into adjacent spectral channels
- Suitable for in vitro and in vivo proliferation studies
- Combine with cytokine secretion, checkpoint marker, memory phenotype

In a congenic adoptive immunotherapy mouse model

- Resting and dividing cells identified after 7-14 days
- Differences in CD4+ and CD8+ division identified
- Homeostatic tissue localisation
- Memory phenotype

Further applications

- CAR T cell studies
- Adoptive immunotherapy of anti-viral T cells
- Allogeneic/autologous stem cell transplant models
- Effects of aging on AT recipients

Acknowledgements

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- Poster #4

Thank you

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