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Dasatinib as a rapid pharmacological ON/OFF switch for T cell bispecific antibody-induced T cell activation and cytokine release

Gabrielle Leclercq[1], Helene Haegel[1], Anneliese Schneider[1], Estelle Marrer-Berger[2], Antje Walz[2], Christophe Boetsch[2], Vesna Pulko[3], Cristiano Ferlini[3], Christian Klein[3]

[1]Pharmaceutical Sciences, Roche Innovation Center Zurich, Schlieren, Switzerland

[2]Pharmaceutical Sciences, Roche Innovation Center Basel, Basel, Switzerland

[3] Oncology Discovery and Translational Area, Roche Innovation Center Zurich, Schlieren, Switzerland

Disclosure

I declare employment and patents with Roche.

Content of the presentation

1. Background:
    - T cell bispecific antibodies-related toxicity risks
    - Dasatinib mode of action
2. Results :
    - Dose-dependent and reversible effect of dasatinib
    - Dasatinib as an on-off switch for TCB-induced T cell activation and cytokine release
3. Take-home messages

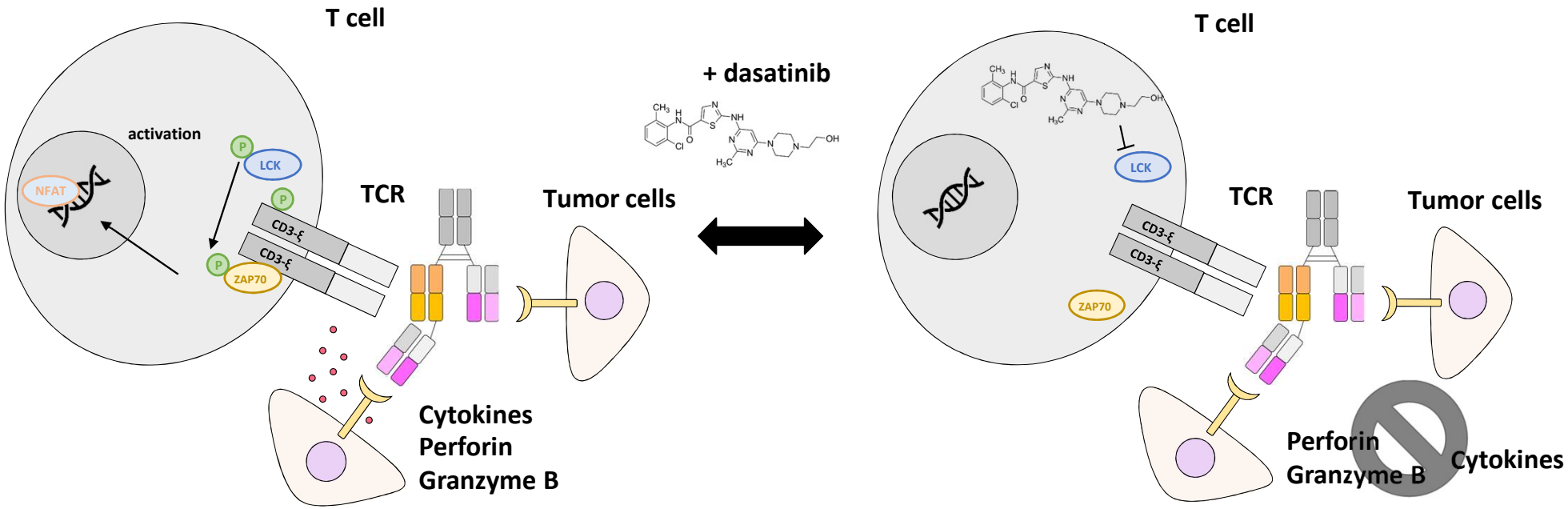
On-target, off-tumor toxicity risks associated with T cell bispecific antibodies (TCBs)

Example of on-target off tumor toxicity risk associated to HLA-A2 WT1-TCB

Illustrations made with Biorender

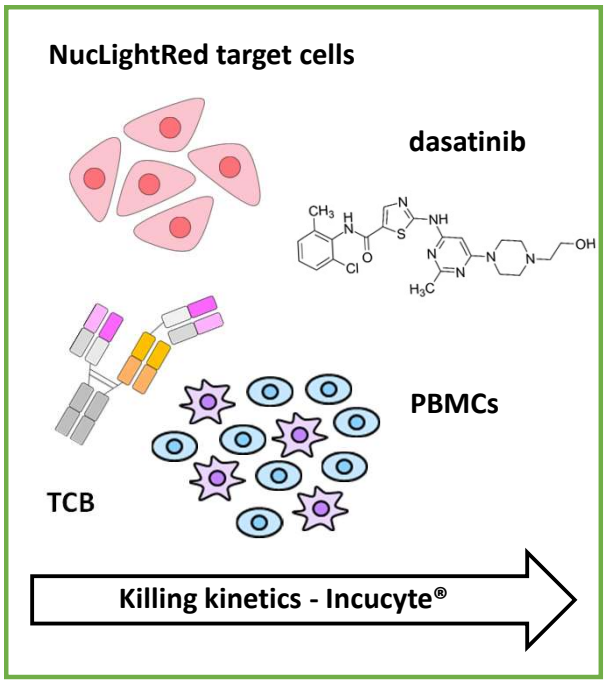
Expression of tumor associated antigen on healthy cells could lead to unwanted T cell activation and potential tissue damage after treatment with TCB.
Recognition of non-WT1 expressing cells presenting homologous peptide on HLA-A2 could lead to killing of healthy cells after treatment with HLA-A2 WT1-TCB.

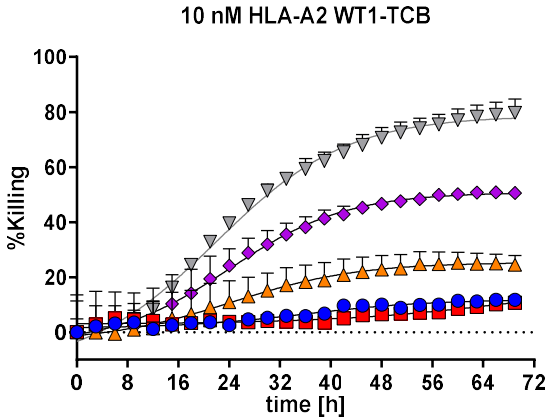
Proposed mode of action of dasatinib



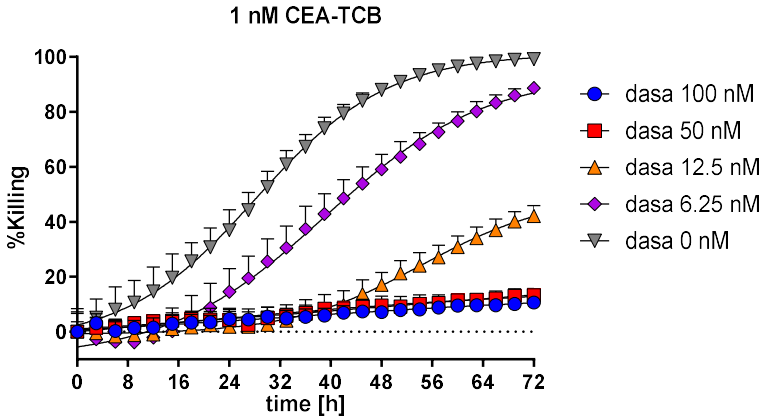
[Re CAR-T: Weber, Lynn et al. 2019 / Mestermann, Giavridis et al. 2019]

Dasatinib prevents TCB-mediated target cell killing at pharmacologically active dose





dasatinib [ng/mL]	dasatinib [nM]	% inhibition
48.8	100	86.5
24.4	50	89.0
6.1	12.5	69.0
3.05	6.25	35.4
0	0	0.0



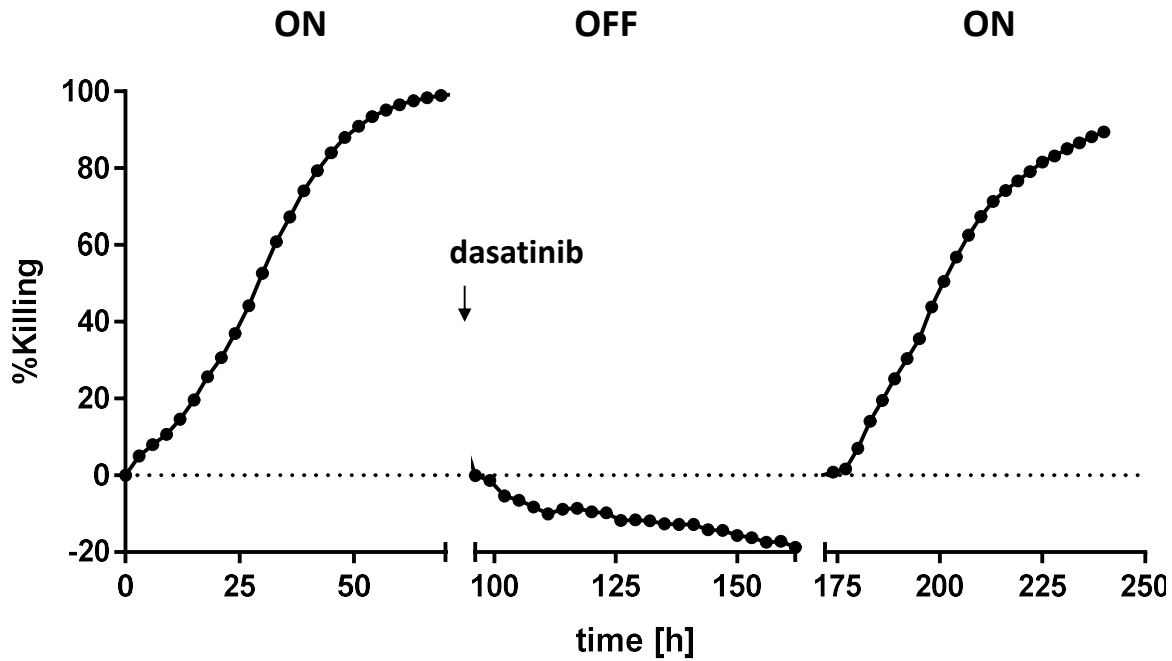
dasatinib [ng/mL]	dasatinib [nM]	% inhibition
48.8	100	90.4
24.4	50	88.2
6.1	12.5	78.2
3.05	6.25	34.4
0	0	0.0

The dose of dasatinib recommended for the patient is 100 mg Q.D. which leads to $\mathbf{C_{min}}$ = 2.61 ng/mL ~ **53 nM** and $\mathbf{C_{max}}$ = 54.6 ng/mL ~ **112 nM***

*Wang et al. 2013

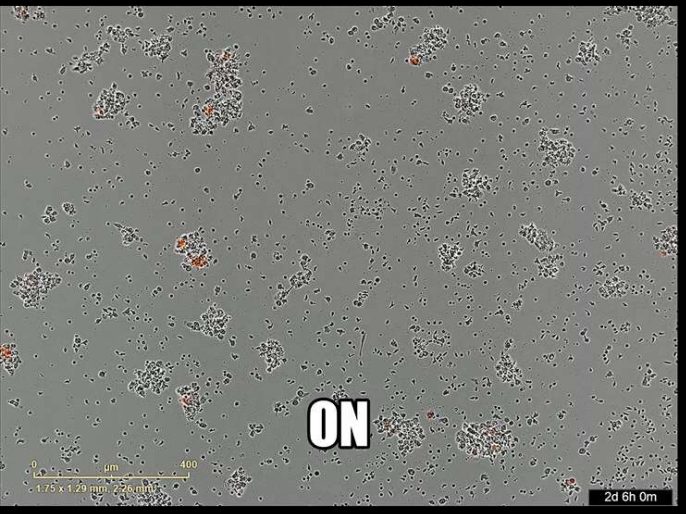
The blockade of TCB-mediated target cell killing by dasatinib is reversible

1 nM CEA-TCB

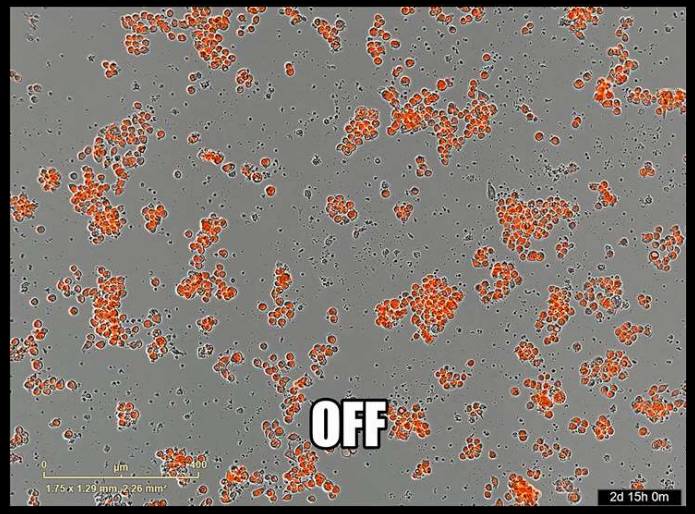


1 nM CEA-TCB

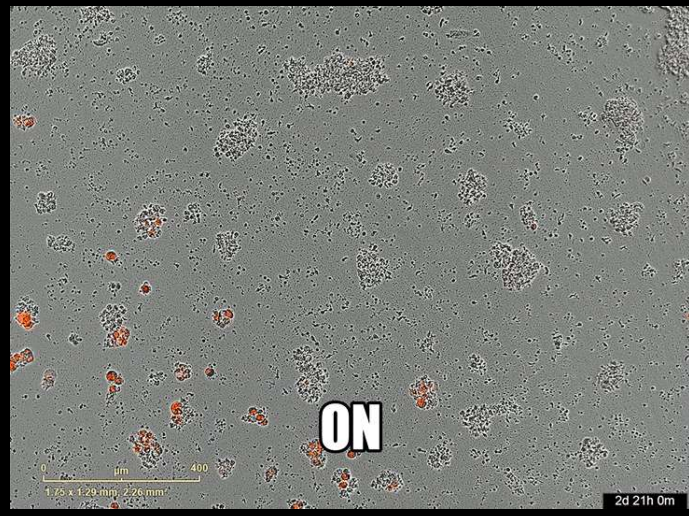
+ 0 nM dasatinib



+ 100 nM dasatinib



+ 0 nM dasatinib



Real time killing of MKN45 NucLightRed cells by CEA-TCB followed by Incucyte®.
Last scan of 1st, 2nd, and 3rd stimulation,
zoom 10x, phase and red, 400ms acquisition time.

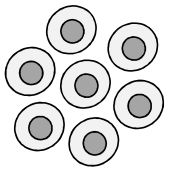
Can dasatinib switch off TCB-activated T cells?

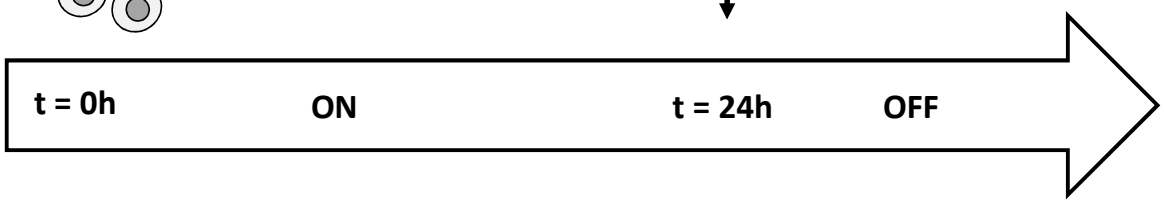
CellTrace™ Violet PBMCs

10 nM HLA-A2 WT1-TCB

100 nM dasatinib

SKM-1 cells





Readouts:

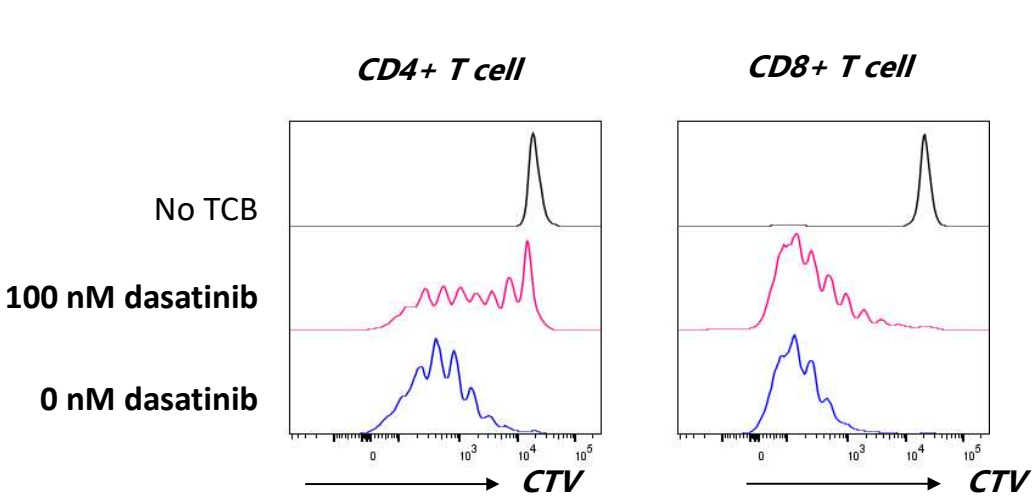
T cell proliferation (144h)

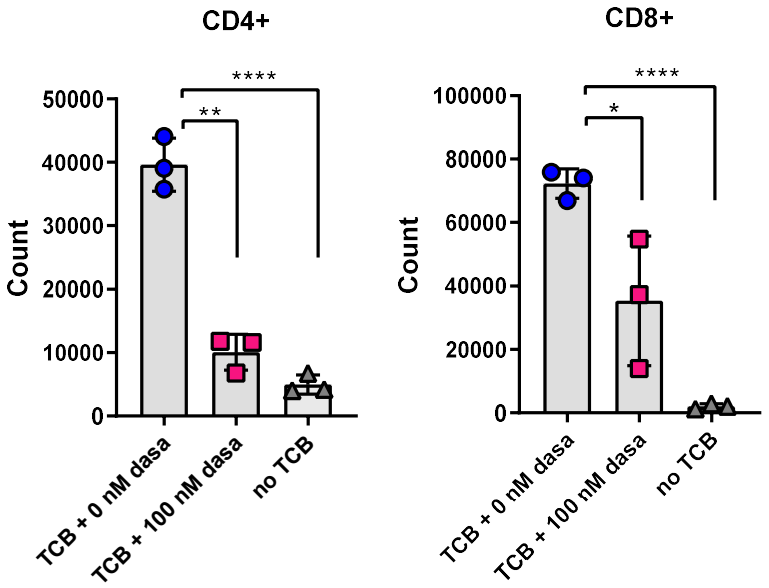
T cell activation (24h and 48h)

Cytokine release (24h and 48h)

Dasatinib reduces T cell proliferation induced by HLA-A2 WT1-TCB in the presence of SKM-1 target cells

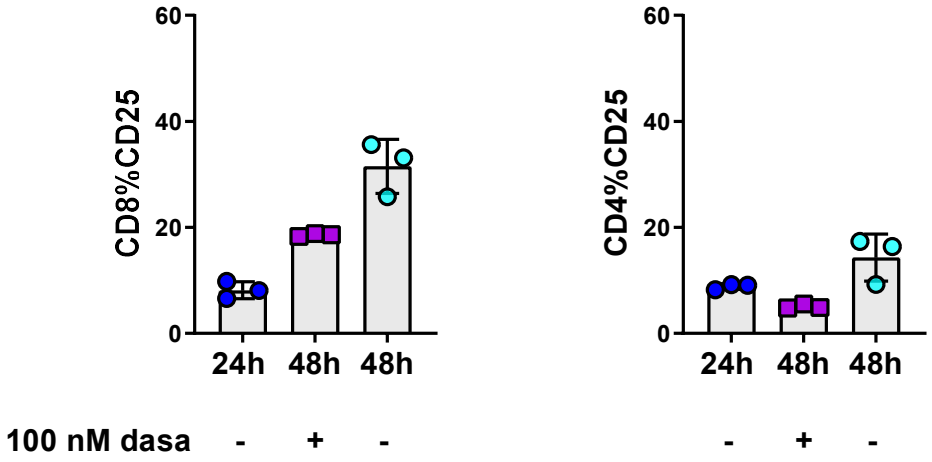
10 nM HLA-A2 WT1-TCB





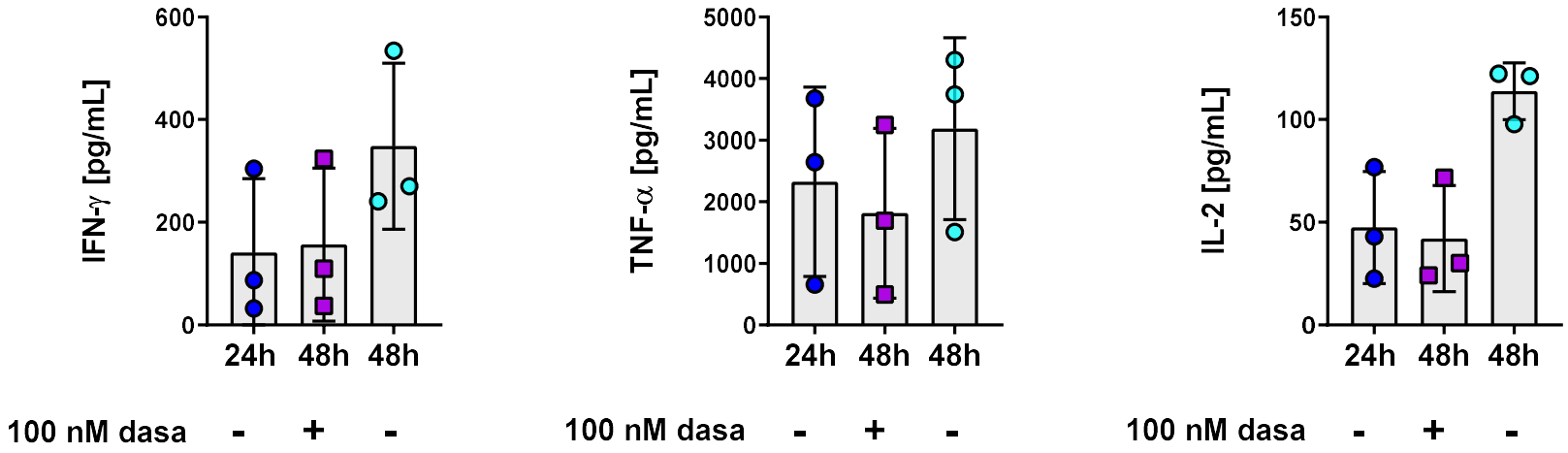
Dilution of the CellTrace™ Violet dye and counts of CD4+ and CD8+ cells was measured by flow cytometry 6 days after the assay start for n= 3 donors.

Dasatinib stops T cell activation induced by HLA-A2 WT1-TCB in the presence of SKM-1 target cells



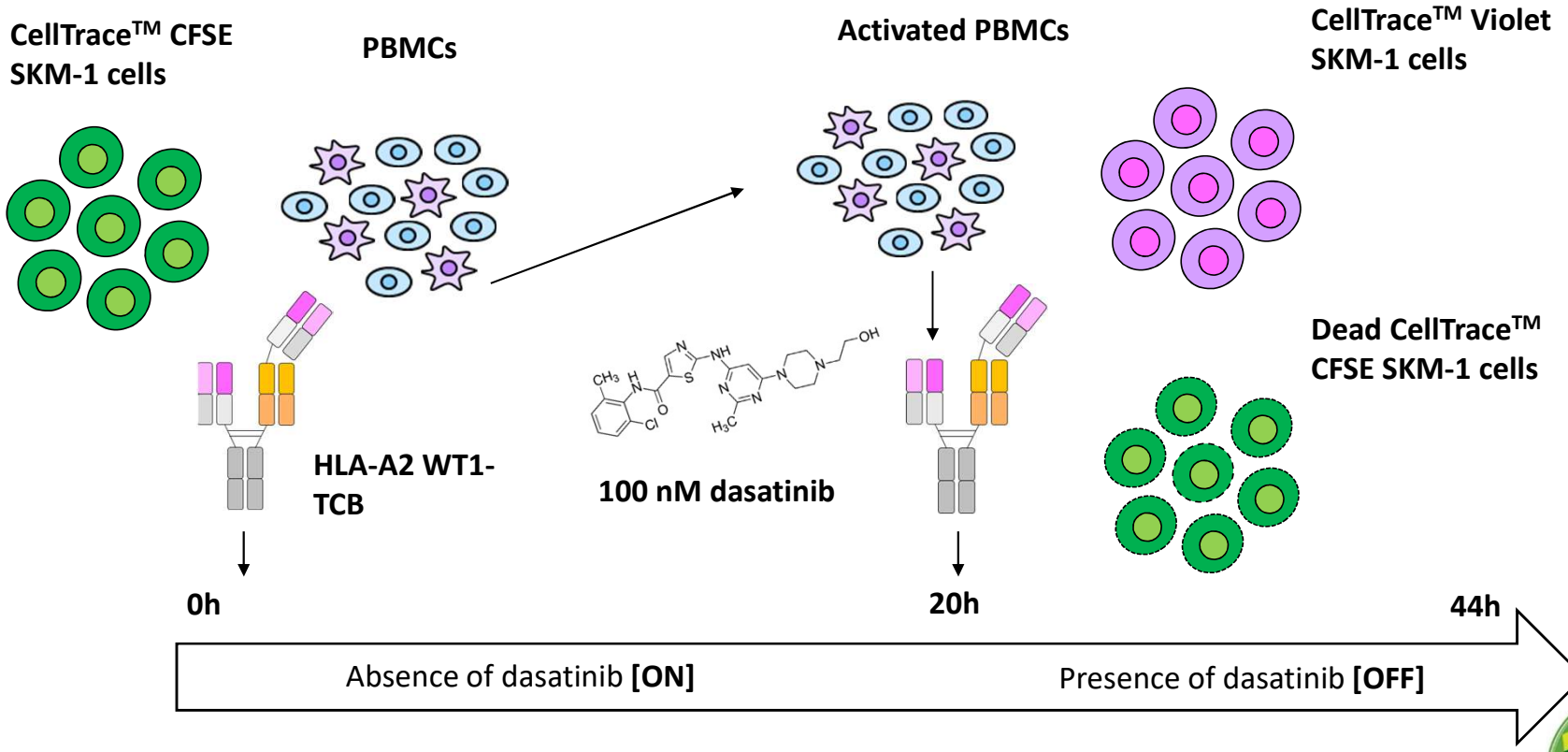
Expression of CD25 on CD4+ and CD8+ T cells was measured by flow cytometry at 24 hours and at 48 hours in the presence and absence of dasatinib for n=3 donors.

Dasatinib stops cytokine release induced by HLA-A2 WT1-TCB in the presence of SKM-1 target cells



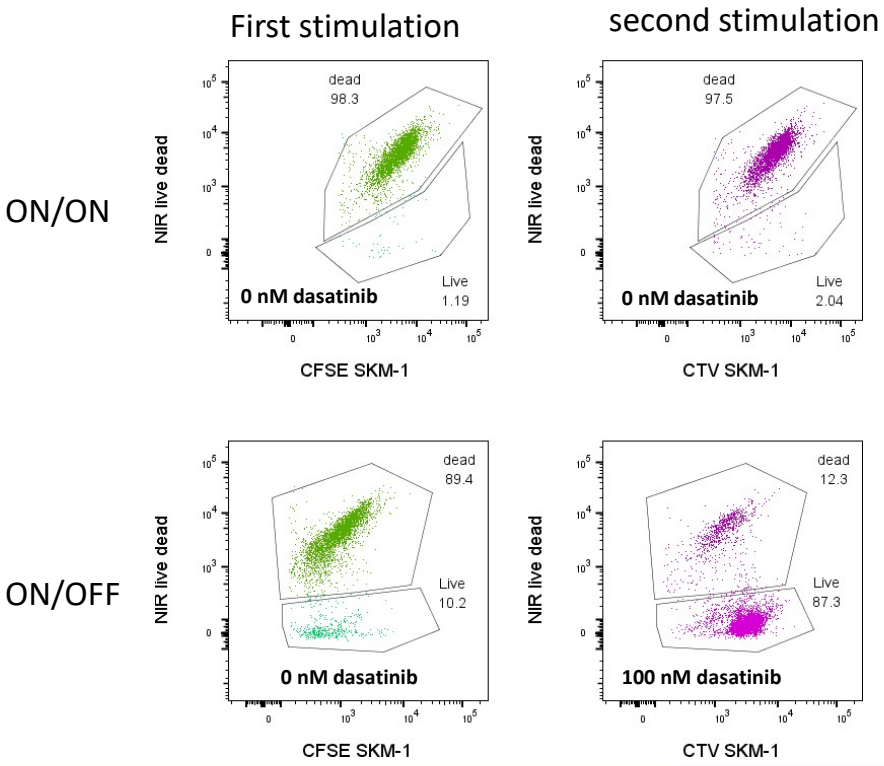
PBMCs were stimulated with 10 nM HLA-A2 WT-1-TCB in the presence of SKM-1 cells. Cytokines were detected in the supernatants using Luminex at 24 hours and at 48h in the presence and absence of dasatinib.

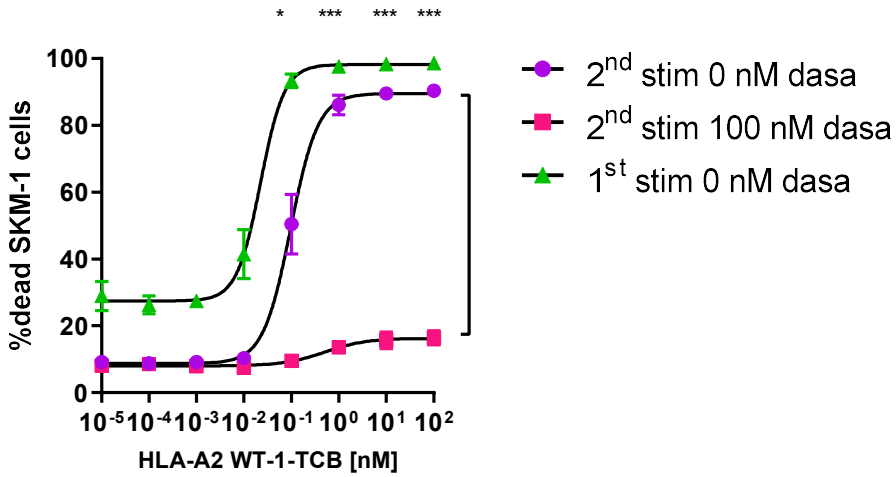
Does dasatinib prevent TCB-mediated target cell killing?



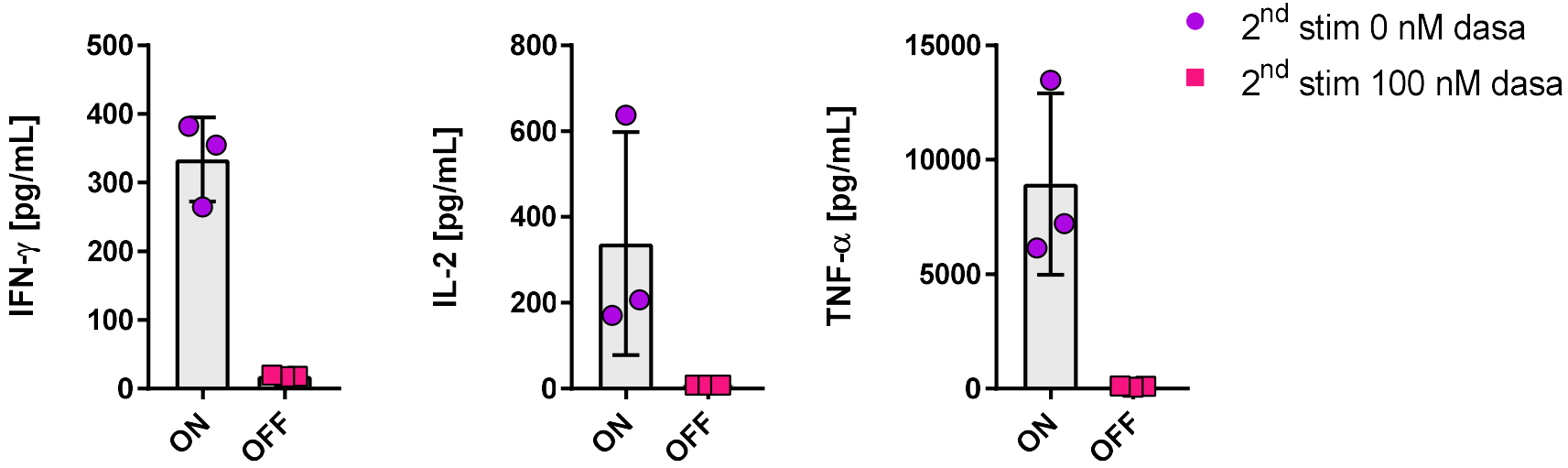
Dasatinib prevents target cell killing upon second TCB stimulation

10 nM HLA-A2 WT-1-TCB





Dasatinib switches off cytokine release by PBMCs upon second TCB stimulation



Cytokines are detected in the supernatant by Luminex 24 hours after second stimulation with 10 nM HLA-A2 WT-1-TCB in the presence and absence of dasatinib.

Take-home messages

It was shown that dasatinib:

- Is effective at pharmacological active dose (100 mg Q.D.)
- Reversibly switches off TCB-activated T cells
- Efficiently stops HLA-A2 WT1-TCB-induced T cell proliferation, activation and cytokine release
- Efficiently prevents HLA-A2 WT1-TCB-induced target cell killing
- Inhibits the release of IL-2, TNF-α and IFN-γ by activated T cells

Dasatinib could be a promising mitigation strategy for off-tumor related toxicities or high grade cytokine release syndrome induced by T cell engaging therapies.
The *in vivo* proof-of concept study is on-going.

Acknowledgment

- Vesna Pulko
- Cristiano Ferlini
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- Sara Colombetti
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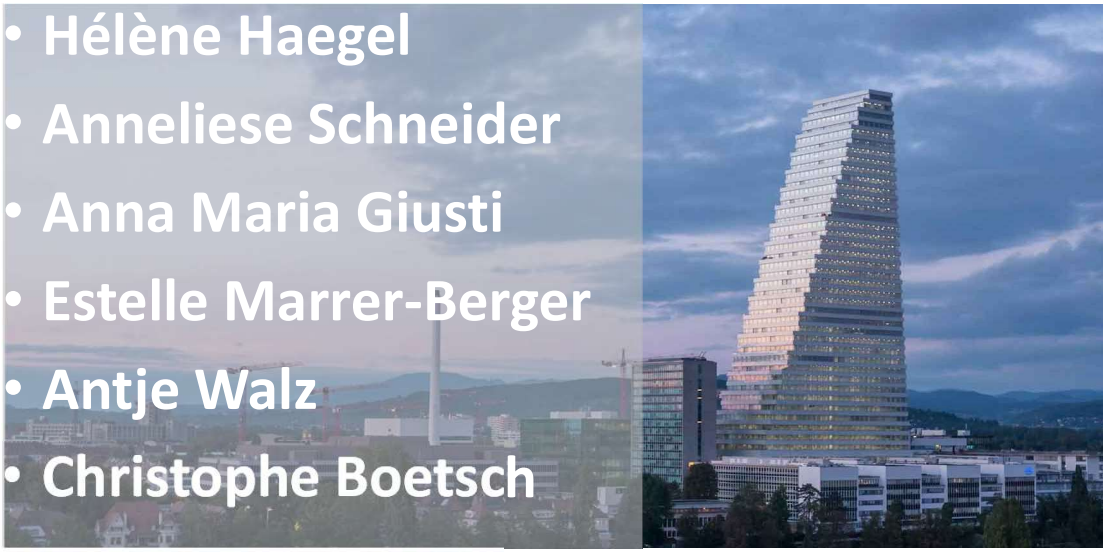
- RICZ colleagues



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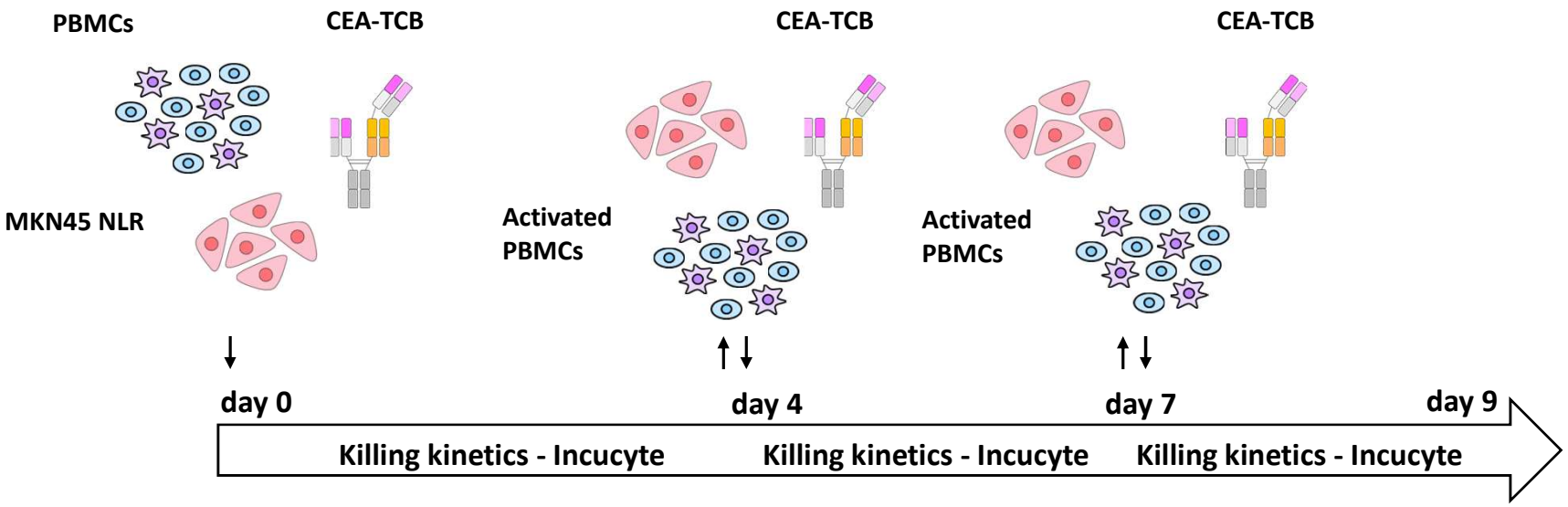
- Antje Walz
- Christophe Boetsch

University of Basel

Prof. Alex Odermatt

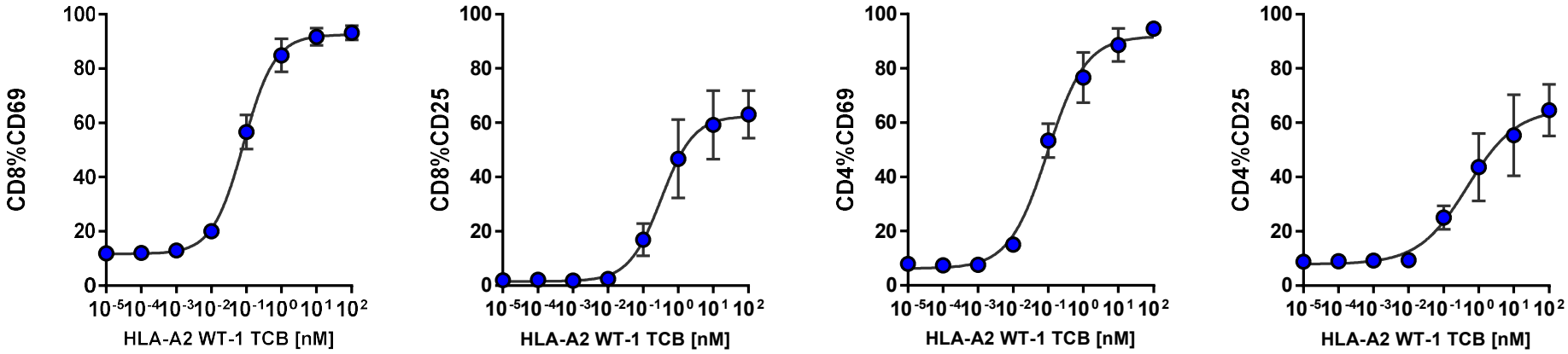
Back-up slides

Is T cell activation blockade with dasatinib reversible?



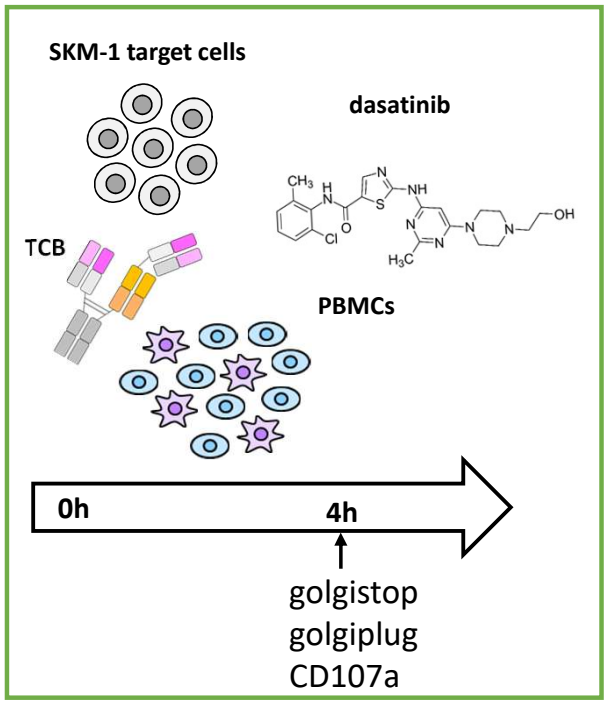
	1st stimulation	2nd stimulation	3rd stimulation	
dasatinib	-	+	-	ON/OFF/ON
	+	-	+	OFF/ON/OFF

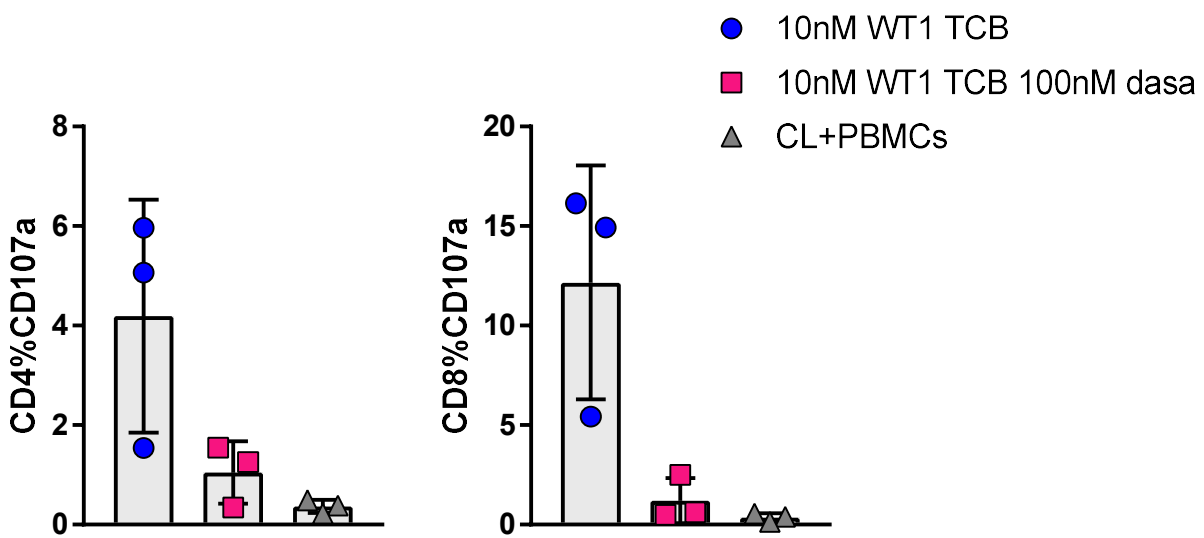
T cells are activated following first TCB stimulation (20 hours)



CD69 and CD25 expression on CD4+ and CD8+ T cell was measured by FACS at 24h.
Data are shown as mean of 3 donors +/- SEM.

Dasatinib prevents TCB-induced T cell degranulation





CD107a expression on CD4+ and CD8+ T cell was measured by FACS at 24h. Data are shown as mean of 3 donors +/- SEM.