

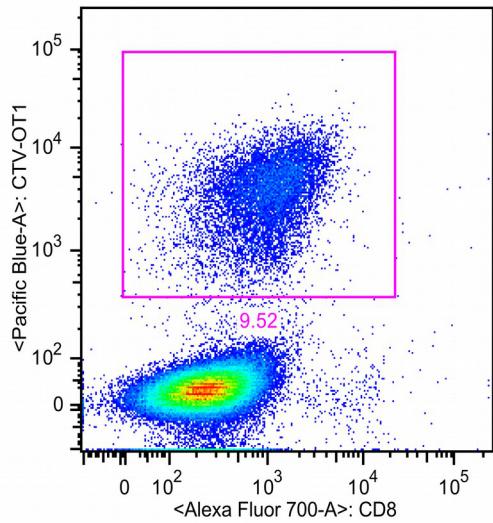
Dataset: 20140920-OT1-dynamics
MIFlowCyt-Compliant Items

| Requirement | Please Include Requested Information |
|--|---|
| 1.1. Purpose | To measure ppERK response of signaling activated mouse CD8 T-cells to inhibitory SRC and inhibitory MEK drugs. |
| 1.2. Keywords | SRC, MEK, MAP-Kinase, CD8, T-Cell |
| 1.3. Experiment variables | Single dose of Dasatinib applied in vitro. |
| 1.4. Organization name and address | Memorial Sloan Kettering Cancer Center, New York, NY, 10065, USA |
| 1.5. Primary contact name and email address | Robert Vogel r.vogel@ibm.com |
| 1.6. Date or time period of experiment | September 2014 |
| 1.7. Conclusions | Applied Dasatinib dose has significantly changed ppERK response vs. untreated control. |
| 1.8. Quality control measures | Concentration of antibodies and other reagents were heavily titrated. Duration of staining, temperature of staining, and buffers used were optimized. Multiple replicates were performed for each experiment – it is part of a series of experiments, the rest of which were published in Vogel et al., Nat. Comm. 7, 12428 (2016) |
| 2.1.1.1. (2.1.2.1., 2.1.3.1.) Sample description | OT-1 primary cells were cultured ex vivo with peptide pulsed APCs from irradiated (3,000 RAD) B10A and B6 mice, respectively. APCs were pulsed with 1 mM SIINFEKL for OT-1 activation prior to irradiation. Cells were purified by Ficoll- Paque gradient centrifugation and given exogenous IL-2 (1 nM) every other day. All cells were cultured at 37 °C and 5% CO ₂ in supplemented RPMI and used for experiments within 7 days of activation. |
| 2.1.1.2. Biological sample source description | RMA-S TAP-deficient T-cell lymphoma cell line was used as APCs for signaling experiments. |
| 2.1.1.3. Biological sample source organism description | Primary splenocytes and lymphocytes were harvested from C57BL/6N (B6; Taconic Farms), and OT-1 TCR transgenic RAG2 / (Taconic Farms), and cultured up to 10 days. Mice were bred, maintained, and euthanized at Memorial Sloan Kettering Cancer Center (MSKCC) in compliance with our animal protocol. The animal protocol was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of the Memorial Sloan Kettering Cancer Center (New York NY). The protocol number is 05-12-031 (last renewal date: 23rd December 2013). |
| 2.1.2.2. Environmental sample location | There were no environmental samples |
| 2.3. Sample treatment description | The ppERK response to SRC and MEK inhibition was measured using primary OT-1 T-lymphocytes activated with RMA-S APCs. RMA-S cells were suspended in culture with 1 nM SIINFEKL peptide for 2 h at 37 °C, 5% CO ₂ , and on a rotator to guarantee mixing. During this time we labelled OT-1 cells with an amine-reactive dye, CTV, according to the manufacturer's protocol (Molecular Probes). This fluorescent tag was used to identify OT-1 cells in silico. We rested the OT-1 cells one hour after CTV staining, and then distributed them in a 96-well v- |

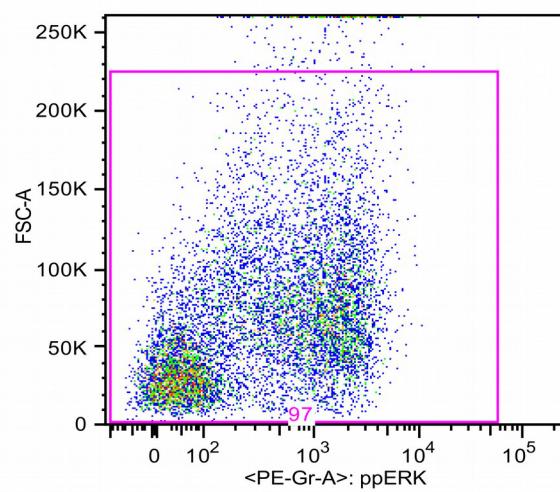
| | | | | | | | | | | | | | |
|--|--|-----------|------|-------------|------|------------|------|-----------|------|-------------|-------|------------|-----|
| | <p>bottom plate. Each well was given various doses of SRC inhibitor and MEK inhibitor and kept at 37 °C for 5 min. Following the 5 min exposure to the inhibitors, we added the peptide pulsed RMA-S (10 RMA-S to 1 OT-1 T cell) and pelleted by centrifugation for 10 s at 460 rcf at room temperature. This step guaranteed that both cell types, OT-1 and RMA-S, came into contact. The cells were allowed to activate for 10 min, followed by fixing on ice in 2% PFA, and then permeabilized and stored in 90% MeOH at 20 °C.</p> | | | | | | | | | | | | |
| 2.4. Fluorescence reagent(s) description | <p>Cells were labelled with primary antibodies against doubly phosphorylated ERK 1/2 (pT202, pY204; clone E10; used at 1:300 dilution), phosphorylated MEK 1/2 (p-S221; clone 166F8; used at 1:100 dilution), phosphorylated STAT5 (p-Y694; clone C11C5; used at 1:200 dilution)—purchased from Cell Signaling Technology (Beverly, Massachusetts)—and polyclonal goat anti-STAT5 (catalogue number sc-835-G; used at 1:200 dilution) purchased from Santa Cruz Biotechnology (Santa Cruz, California). Secondary antibodies tagged with fluorescent molecules include PE conjugated donkey anti-mouse (catalogue number 715-116-151), APC conjugated donkey anti-mouse (catalogue number 715-136-151), FITC conjugated donkey anti-rabbit (catalogue number 711-097- 003), and Alexa Fluor 647 conjugated donkey anti-goat (catalogue number 705-605-147) were all purchased from Jackson ImmunoResearch (West Grove, Pennsylvania; used at 1:200 dilution). In addition, Brilliant Violet 421 donkey anti-rabbit polyclonal antibody was purchased from BioLegend (San Diego, California; catalogue number 406410; used at 1:200 dilution). Surface markers CD8a (clone 53-6.7; used at 1:200 dilution) and CD4 (clone RM4-5; used at 1:300 dilution) tagged to fluorescent molecules were purchased from Tonbo biosciences (San Diego, California).</p> | | | | | | | | | | | | |
| 3.1. Instrument manufacturer | BD Biosciences | | | | | | | | | | | | |
| 3.2. Instrument model | LSRII (Joe Sun Lab, MSKCC) | | | | | | | | | | | | |
| 3.3. Instrument configuration and settings | <p>Parameter voltages:</p> <p>FSC: 450 SSC: 225 FITC (pMEK): 550 A-700 (CD8): 550 PE (ppERK): 425 Pacific Blue (CTV): 250</p> <p>Laser settings:</p> <table> <tbody> <tr> <td>LASER1ASF</td> <td>1.08</td> </tr> <tr> <td>LASER1DELAY</td> <td>0.00</td> </tr> <tr> <td>LASER1NAME</td> <td>Blue</td> </tr> <tr> <td>LASER2ASF</td> <td>1.00</td> </tr> <tr> <td>LASER2DELAY</td> <td>99.99</td> </tr> <tr> <td>LASER2NAME</td> <td>Red</td> </tr> </tbody> </table> | LASER1ASF | 1.08 | LASER1DELAY | 0.00 | LASER1NAME | Blue | LASER2ASF | 1.00 | LASER2DELAY | 99.99 | LASER2NAME | Red |
| LASER1ASF | 1.08 | | | | | | | | | | | | |
| LASER1DELAY | 0.00 | | | | | | | | | | | | |
| LASER1NAME | Blue | | | | | | | | | | | | |
| LASER2ASF | 1.00 | | | | | | | | | | | | |
| LASER2DELAY | 99.99 | | | | | | | | | | | | |
| LASER2NAME | Red | | | | | | | | | | | | |

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| | <table> <tbody> <tr><td>LASER3ASF</td><td>1.27</td></tr> <tr><td>LASER3DELAY</td><td>66.41</td></tr> <tr><td>LASER3NAME</td><td>UV</td></tr> <tr><td colspan="2"> </td></tr> <tr><td>LASER4ASF</td><td>1.05</td></tr> <tr><td>LASER4DELAY</td><td>130.04</td></tr> <tr><td>LASER4NAME</td><td>Green</td></tr> <tr><td colspan="2"> </td></tr> <tr><td>LASER5ASF</td><td>1.06</td></tr> <tr><td>LASER5DELAY</td><td>34.67</td></tr> <tr><td>LASER5NAME</td><td>Violet</td></tr> </tbody> </table> | LASER3ASF | 1.27 | LASER3DELAY | 66.41 | LASER3NAME | UV | | | LASER4ASF | 1.05 | LASER4DELAY | 130.04 | LASER4NAME | Green | | | LASER5ASF | 1.06 | LASER5DELAY | 34.67 | LASER5NAME | Violet |
| LASER3ASF | 1.27 | | | | | | | | | | | | | | | | | | | | | | |
| LASER3DELAY | 66.41 | | | | | | | | | | | | | | | | | | | | | | |
| LASER3NAME | UV | | | | | | | | | | | | | | | | | | | | | | |
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| LASER4ASF | 1.05 | | | | | | | | | | | | | | | | | | | | | | |
| LASER4DELAY | 130.04 | | | | | | | | | | | | | | | | | | | | | | |
| LASER4NAME | Green | | | | | | | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | | | | | | | |
| LASER5ASF | 1.06 | | | | | | | | | | | | | | | | | | | | | | |
| LASER5DELAY | 34.67 | | | | | | | | | | | | | | | | | | | | | | |
| LASER5NAME | Violet | | | | | | | | | | | | | | | | | | | | | | |
| 4.1. List-mode data files | <p>All data for this experiment can be found in</p> <p>https://github.com/AmirErez/BimodalLogspaceCytA/tree/master/Data/20140920-OT1-dynamics</p> <p>In particular, the list-mode file <u>Specimen_005_E5_E05.fcs</u> and accompanying compensation single stain controls.</p> | | | | | | | | | | | | | | | | | | | | | | |
| 4.2. Compensation description | Compensation samples were prepared the same way as the sample cells. See compensation plots below. | | | | | | | | | | | | | | | | | | | | | | |
| 4.3. Data transformation details | Explained in main article | | | | | | | | | | | | | | | | | | | | | | |
| 4.4.1. Gate description | See figures below for detailed visual description. Cells were gated for CD8+CTV+ with extremely high FSC outliers excluded. | | | | | | | | | | | | | | | | | | | | | | |
| 4.4.2. Gate statistics | See figures below | | | | | | | | | | | | | | | | | | | | | | |
| 4.4.3. Gate boundaries | See figures below | | | | | | | | | | | | | | | | | | | | | | |

Gating strategy:



Ungated
Specimen_005_E5_E05.fcs
Event Count: 100913



CTV+CD8+
Specimen_005_E5_E05.fcs
Event Count: 9604

Compensation (spillover matrix): single stain on vertical axis, spilled-into channels in horizontal axis. This plot was produced with FlowJo 9.9 (OSX, default settings).

