# Immunomodulation of Primary Human T Cell Activation Using High-throughput Screen of FDA-approved Small Molecule Drugs

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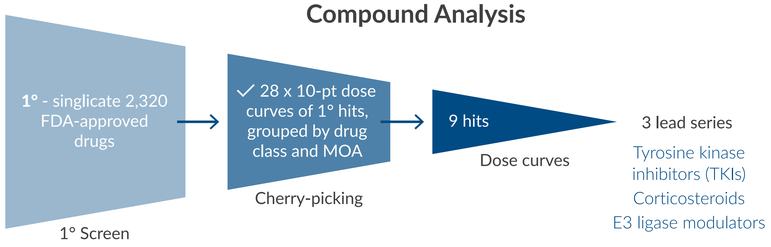
## KEY FINDING

**By combining IL-2 secretion and viability assays, our screening platform accurately identifies multiple members of active compound classes and differentiates immunomodulatory or toxic activity for T cells.**

## ABSTRACT

Immunotoxicity and immunomodulation are frequent on- and off-target properties of therapeutics. The incorporation of immune function assays into structure-activity relationship (SAR) studies can provide valuable guidance for drug development, whether for drug safety and/or efficacy. In this high-throughput screen (HTS), we focus on the health and function of primary human T cells through utilization of an AlphaLISA IL-2 assay for IL-2 secretion and CellTiter-Glo® assay for cell survival and proliferation. When stimulated via the T cell receptor, T cells will begin expansion and secretion of the cytokine IL-2.

Incorporation of liquid handling automation allows for miniaturization of this primary cell-based assay to the 384-well format with improved throughput and reduction in material costs. Up to 28 × 10-point dose curves can be performed per 384-well plate in singlicate with replicates produced across multiple plates or 9 × 10-point dose curves performed in triplicate on a single 384-well plate. By utilizing cryopreserved peripheral blood mononuclear cells (PBMCs), assays can be regulated for batch-to-batch consistency and data turn around reduced to 48 hours from drug treatment.




## HIGH-THROUGHPUT 1° SCREENING METHODS AND RESULTS

Our objective was to optimize our workflow for performance of a HTS of 2,320 FDA-approved small molecule drugs using an IL-2 AlphaLISA assay to detect changes in T cell activation. We sought to test whether modulation of IL-2 secretion by known drugs could be measured and characterized for future SAR studies. We developed workflows for both 1° screening at a single drug concentration, to be followed by cherry-picking of hits for dose curves and cytotoxicity evaluation, for identification of compounds that are cytotoxic *versus* immunomodulatory.

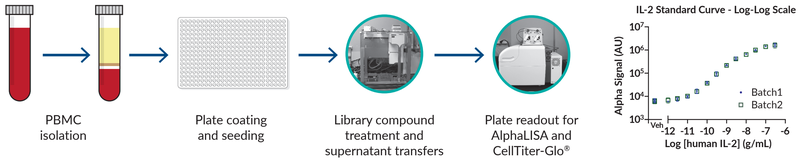



**FIGURE 1** – Workflow for cell isolation, treatment, and readout.

- Isolate human PBMCs from whole blood.
- Activate T cells by seeding on αCD3/αCD28-coated 384-well plates.
- Treat with 2,320 FDA-approved compounds at 250 nM from 8 × 384-well library stock plates in DMSO.
- Staurosporine (SP) was a control for IL-2 inhibition and cytotoxicity.
- Incubate for 48 hours and transfer supernatant for IL-2 detection by AlphaLISA.

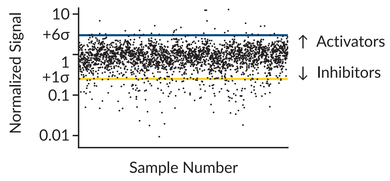



**FIGURE 2** – Dot plot of 1° screen hits.

Cutoffs for IL-2 secretion activators were set relative to the vehicle control (0.025% DMSO) and the cutoff for inhibitors set relative to the negative control (250 nM SP). The sample values are normalized to the average signal across the full dataset.

| Compound Class | Identified Members from 1° Screen Hits |
|---|---|
| Tubulin inhibitors | colchicine, vinorelbine, vinblastine |
| E3 ligase modulators | lenalidomide, pomalidomide |
| TKIs | dasatinib, ponatinib, masitinib, erlotinib, regorafenib, bosutinib, dovitinib, foretinib, ibrutinib, (R)-crizotinib (but not imatinib, nilotinib) |
| Corticosteroids | halobetasol, triamcinolone, fluocinonide, clobetasol, mometasone, dexamethasone |
| Anthracyclines | daunorubicin, idarubicin (but not epirubicin) |

**TABLE 1** – Drug classes identified by multiple hits in the 1° screen.

The HTS highlighted several classes of compounds that affected IL-2 secretion by T cells. Cutoff values for activation were set relative to DMSO vehicle controls and identified 1.0% of the FDA-approved library as IL-2 secretion stimulators at 250 nM treatment. Cutoff values for inhibition were set based on the negative control and identified 4.4% of the library as inhibitors of IL-2 production. IL-2 secretion activators are highlighted in blue and inhibitors are highlighted in yellow.

## HIT PICKING FOR REPRODUCIBILITY AND EVALUATION OF POTENCY

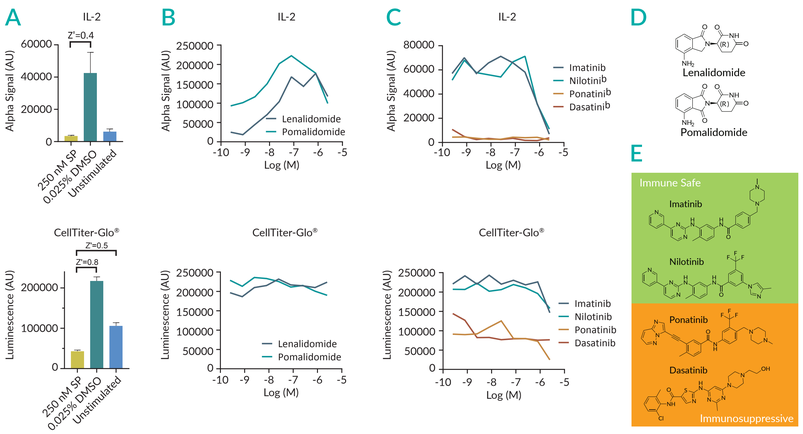



**FIGURE 3** – Singlicate dose curves of cherry-picked hits based on drug class.

We verified the activity of 28 of the top activators and inhibitors grouped by drug class from the 1° screen to eliminate false positives. **A)** Control values from singlicate dose curve readouts. AlphaLISA Z' ranged from 0.4-0.6 during optimization and testing. CellTiter-Glo® Z' ranged from 0.7-0.8. **B)** From the E3 ligase modulators, the doses tested did not result in cell death. The effect at 250 nM in the 1° screen is reproducible. **C)** TKIs showed member-specific suppression of T cell activation, presenting opportunity for quantitative evaluation of SAR. **D)** E3 ligase modulator compound structures. **E)** TKI compound structures.

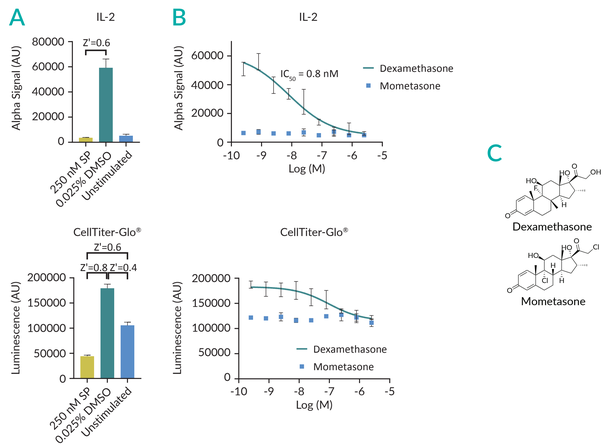



**FIGURE 4** – Dose curves of verified hit compounds.

We performed dose curves in triplicate for nine compounds with verified activity in the 1° screen. **A)** Control values from singlicate dose curve readouts. **B)** We measured the inhibitory concentration at 50% inhibition ($IC_{50}$) for dexamethasone against IL-2 secretion at 0.8 nM. Typical values of glucocorticoid receptor activity for dexamethasone are low nM. Mometasone is known to bind the glucocorticoid receptor 22 times stronger than dexamethasone.[1] **C)** Corticosteroid compound structures.

## CONCLUSION

- The use of primary T cells gives a more direct *in vivo* comparison of small molecule drug activity for validation of mechanism of action for therapy.
- IL-2 secretion (T cell function) paired with cytotoxicity assays (T cell survival) can be used to differentiate small molecule immunotoxicity or immunomodulation of T cells, as well as direct cell killing mechanisms.
- T cell activators included certain tubulin inhibitors as well as E3 ligase modulators.[2]
- IL-2 suppressors that were not cytotoxic included corticosteroids and certain TKIs.
- Some TKIs have off-target effects on T cell receptor signaling at the tested dose, which highlighted immune safe members of this compound group that were tested during cherry picking for verification.[3]

**References**

1. Mometasone furoate DrugBank DB14512. go.drugbank.com/drugs/DB14512.
2. Gandhi, A.K., Kang, J., Havens, C.G., *et al.* Immunomodulatory agents lenalidomide and pomalidomide co-stimulate T cells by inducing degradation of T cell repressors Ikaros and Aiolos via modulation of the E3 ubiquitin ligase complex $CRL4^{CRBN}$. BJ Haem. **164(6)**, 811-821 (2014).
3. Lamble, A.J., Kosaka, Y., Laderas, T., *et al.* R. Reversible suppression of T cell function in the bone marrow microenvironment of acute myeloid leukemia. *Proc. Natl. Acad. Sci. USA.* **117(25)**, 14331-14341 (2020).

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