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EpiScreen® 2.0 Time Course Assay: A Sensitive & Data-Rich Tool for Pre-Clinical Immunogenicity Testing of Biologic

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PURPOSE

The purpose of this study was to develop a next-generation immunogenicity assay that does not compromise sensitivity, while delivering data on specificity and mechanism of action. The result is an improved assay which increases confidence in pre-clinical immunogenicity risk assessment data and lead selection.

METHODS

PBMCs were isolated from healthy community donor leukopaks. A cohort of 50 donors was selected and HLA typed. Each test sample (Herceptin®, Abciximab, Atezolizumab, ATR-107, CEFT, KLH) was added to the PBMCs, and cultures were incubated for a total of 8 days at 37°C with 5% CO_2. Cells were analyzed by flow cytometry at 3 time points. Cells were stained for CD3, CD4, CD25, CD134 (OX-40) and viability, while EdU uptake was the readout for proliferation (Figure 1).

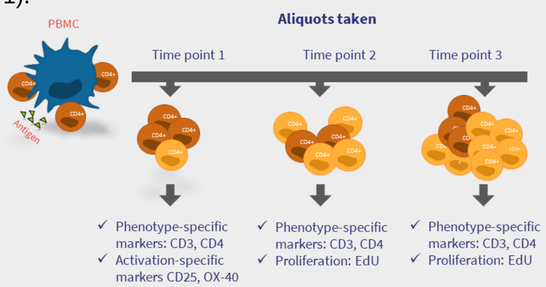

Figure 1. Schematic of EpiScreen® 2.0 assay

RESULTS

EpiScreen® 2.0 specifically assessed $CD4^+$ T cell proliferation via flow cytometry, providing more relevant information than a bulk readout. Proliferation assessment via EdU incorporation has a wider assay window than the traditional [3H]-thymidine method, offering better ranking of immunogenicity. Furthermore, the activation-induced markers (AIM) CD25 and OX-40 help to pinpoint samples with higher risk of immunogenicity (Figures 2, 3, 4 and Table 1). This new assay further allows the monitoring of other cell populations, such as $CD8^+$ activation, thereby providing insights into the mechanism of action – particularly useful for gene therapy where vectors can be cross-presented on MHC-I.

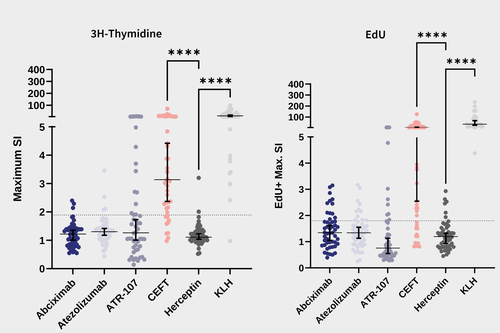

Figure 2. Box and whisker plot showing maximum proliferation SI obtained on PBMCs (non-$CD8^+$ depleted) over the EpiScreen® 2.0 Time Course assay in response to test samples. Left, [3H]-Thymidine readout. Right, EdU readout on $CD4^+$ T cells. One-way ANOVA, followed by Friedman's post-test was used for statistical analysis. ****p <0.0001 (n=50). The dotted lines represent the threshold for a positive response (SI ≥1.9 for 3H-Thymidine; SI ≥1.8 for EdU).

Proliferation and activation responses in PBMC (non-$CD8^+$ depleted)				
Sample	**% Response ^{3}H-Thy**	**% Response EdU**	**% Response AIM**	**Expected ADA (%)**
Abciximab	6	24	14	6-44
Atezolizumab	8	22	12	13-36
ATR-107	32	20	32	76 (37.5*)
CEFT	80	76	82	70-90$^+$
Herceptin®	4	12	10	10
KLH	98	100	100	90-100$^+$

Table 1. EpiScreen® 2.0 assay proliferation and activation responses. *Reported response in vitro, $^+$Expected assay response rates are reported for CEFT and KLH.

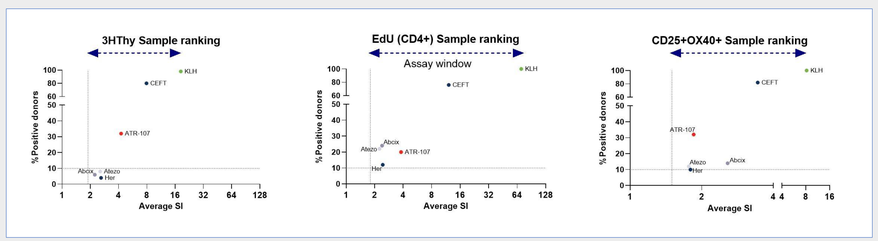

Figure 3. EpiScreen® 2.0 assay performance. T cell response ranking of samples in a 50-donor Time Course cohort. Left, [3H]-Thymidine uptake ranking; Middle, EdU uptake ranking: Right, CD25/OX-40 (AIM) ranking. The dotted lines represent the threshold for a positive response (% positive donors >10% (based on Herceptin® ADA) and SI ≥1.9 for 3H-Thymidine, SI ≥1.8 for EdU and SI ≥1.5 for AIM). The blue dotted arrows indicate the assay window for [3H]-Thymidine and EdU readouts.

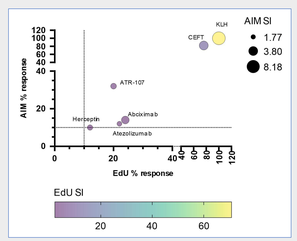

Figure 4. Multiple variable analysis bubble plot: AIM response rate (Y axis), EdU response rate (X axis), SI from AIM positive responses (dot size) and SI from EdU proliferation positive responses (color).

CONCLUSIONS

This assay presents a new and much improved pre-clinical immunogenicity testing method for biologics, which is **sensitive, specific, data-rich, MoA-reflective and customizable.**

The assay is **sensitive** compared to traditional [3H]-Thymidine methods and **specific** to monitor $CD4^+$ T cell proliferation. **Data-rich** complementary read-outs for proliferation and activation markers are **customizable and MoA-reflective,** with options to modify and extend cell-surface markers to monitor other cell populations or activation mechanisms, or to add cytokines analysis by Luminex xMAP or FluoroSpot. The use of a well characterised HLA-typed, HTA regulated Leukopak cell bank allows **repeatable** assays.

REFERENCES

Stempels, F.C. et al., 2022. A sensitive and less cytotoxic assay for identification of proliferating T cells based on bioorthogonally-functionalized uridine analogue. *Journal of Immunological Methods* 502: 113228

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