High-throughput screening of small molecules targeting FoxP3 in regulatory T cells for cancer treatment

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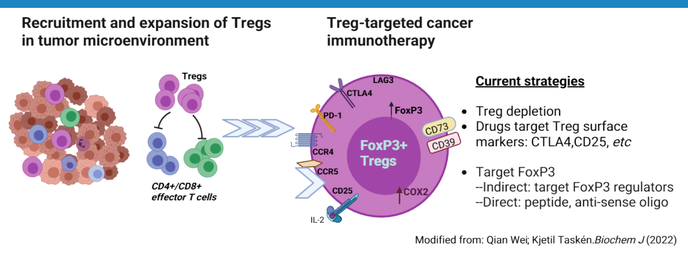
OVERVIEW

- **Small molecule drug screen**
- **High-throughput flow cytometry**
- **FoxP3⁺ Treg**
- **Cancer immunotherapy**

INTRODUCTION



Modified from: Qian Wei, Kjetil Taskén.*Biochem J* (2022)

Aim of the project:
Identify novel small molecules that can directly target FoxP3

- Targeting Tregs is a popular therapeutic strategy in drug discovery to block of anti-tumor immune evasion mechanisms, due to its suppressive function on effector T cells that facilitates tumor cell proliferation.
- FoxP3 is the key lineage-defining transcription factor in Tregs and regulates Treg functions, turning out to be an effective strategy for drug development.
- Here we show a well-established phenotypic high-throughput drug screen on FoxP3 and candidates validation.

METHODS

STEP 1:High-throughput drug screen on FoxP3 regulation

PROCEDURES

--Preparation of human primary CD3⁺ T cells from healthy donor blood)

--Incubation with compounds at 10μM, in 384-well plate

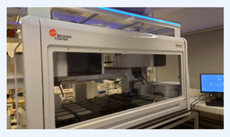
--Flow antibody staining by automated liquid handling system
- Fixation
- Permeabilization
- CD4 and FoxP3 antibody staining

--High-throughput flow cytometry

INSTRUMENTS

CERTUS FLEX Micro Dispenser
--cell distribution into 384-well plate

Echo 550 acoustic dispenser
--compound plate printing
--antibody dispensing

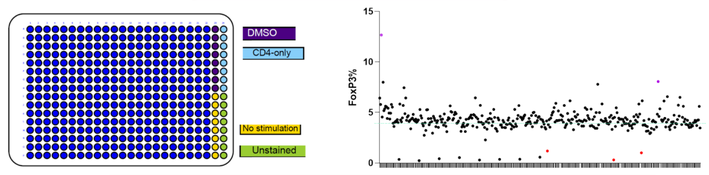


Beckman i7 automated workstation
--flow cytometry antibody staining (liquid handling)

IntelliCyt iQue Screener PLUS
--high-throughput flow cytometry

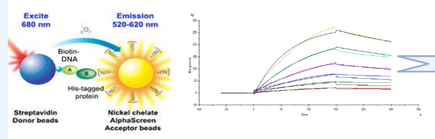
STEP 2: Candidates selection

DATA ANALYSIS



Compounds up- or down-regulate FoxP3

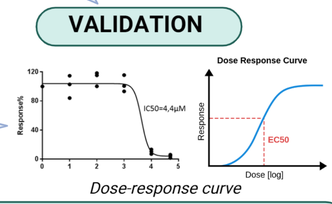
SECONDARY SCREEN



AlphaScreen *Biacore SPR*

--Select compounds that can directly bind FoxP3 or affect FoxP3-DNA binding

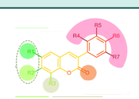
VALIDATION



Dose-response curve

Establish a library of compounds with similar structures

--in silico prediction
--structure-activity relationship (SAR)

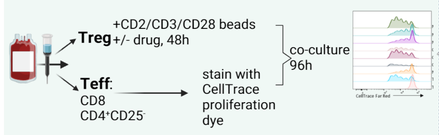


STEP 3: Functional validation

FOXP3 DOWN-REGULATORS

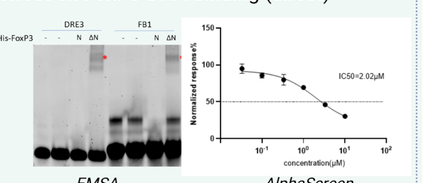
1.*In vitro* functional analysis

--Treg suppression assays



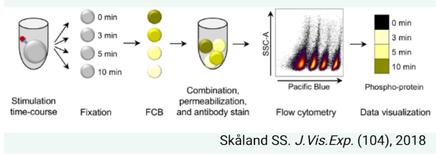
2.Discovery of mechanism of drugs on FoxP3 regulation

--effect on FoxP3-DNA binding (direct)



EMSA *AlphaScreen*

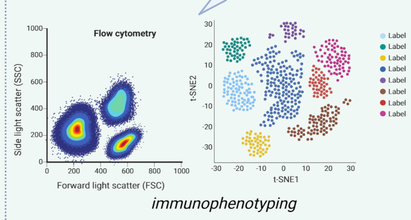
--effect on signaling pathways in Treg(indirect)



Skåland SS. *J.Vis.Exp.* (104), 2018

3.*In vivo* validation

--mouse syngenic tumor model



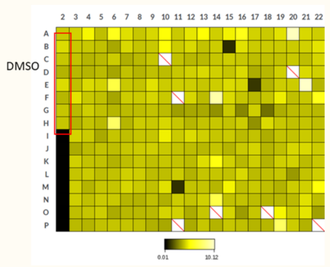
immunophenotyping

--Further application in cancer patients

RESULTS

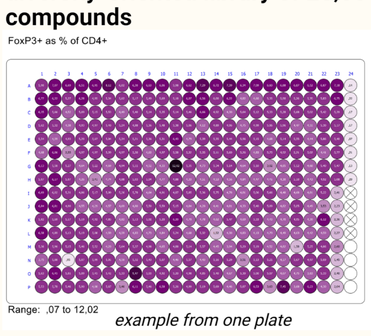
FIGURE 1. Drug screen setup and candidates selection

A. Screen of 1,522 approved drugs



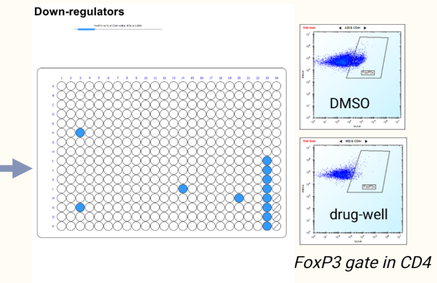
4 candidates that can down-regulate FoxP3 are under functional evaluation *ex vivo/in vitro* and *in vivo*

B. Expand to screen a large diversity-oriented library of 28,500 compounds



example from one plate

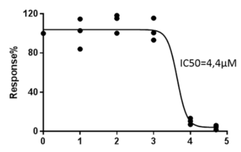
Potential FoxP3 down-regulators will be selected for further validations



FoxP3 gate in CD4

FIGURE 2. Identification of a group of compounds that can down-regulate FoxP3

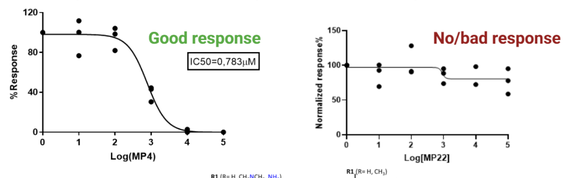
A. Discovery of an anti-malaria drug that can down-regulate FoxP3



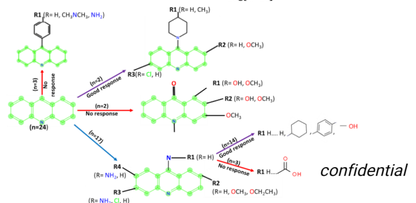
Response%= FoxP3% in CD4⁺ T cells normalized to DMSO control

B. 27 compounds with similar structures were searched by *in silico* prediction and validated

--14 compounds with similar or lower IC50
--13 compounds with no response or bad response on FoxP3



C. SAR conclusion

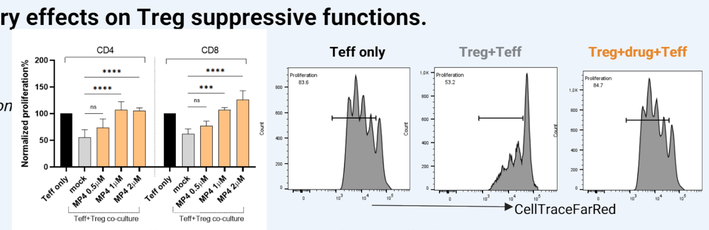


confidential

FIGURE 3. Functional validation of one promising candidate

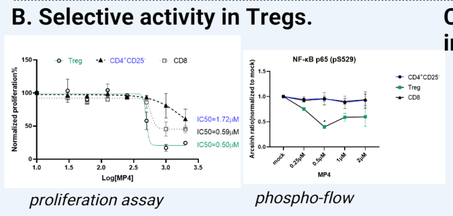
A. Inhibitory effects on Treg suppressive functions.

Treg suppression assay



**normalized response: proliferation of Teff in co-culture with Treg normalized to Teff only (n=3)*

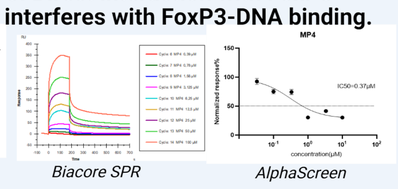
B. Selective activity in Tregs.



proliferation assay

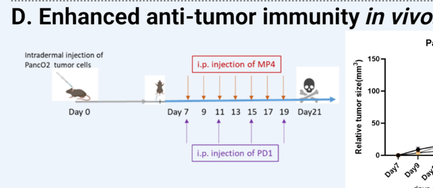
phospho-flow

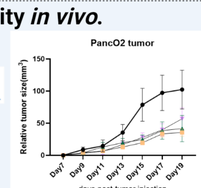
C. Direct interaction with FoxP3 interferes with FoxP3-DNA binding.

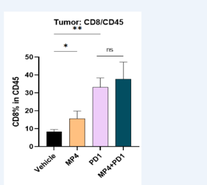


Biacore SPR *AlphaScreen*

D. Enhanced anti-tumor immunity *in vivo*.







CONCLUSIONS

1. We established a phenotypic high-throughput small molecule drug screen on FoxP3 expression using human primary T cells and successfully screened up to 30,000 compounds.
2. With the screen of approved drug library, we could identify potential FoxP3 down-regulators and validate similar compounds based on *in silico* prediction to conclude the SAR information for FoxP3 regulation.
3. The most effective compound was confirmed by functional assays and evaluated in mouse tumor model, revealing enhanced anti-tumor immunity

Importantly, our project proved the feasibility to screen small molecules targeting FoxP3⁺ Tregs, supporting as an alternative and effective approach for anti-cancer drug discovery. Identification of compounds with direct binding on FoxP3 as well as effects on FoxP3-DNA binding and down-stream gene regulation is the main effort in the future, aiming for drugs with high specificity on FoxP3⁺ Tregs and minimum side effects.

ACKNOWLEDGEMENT

@TaskenLab

OSLO UNIVERSITY HOSPITAL | MATRIX NORWEGIAN CENTRE FOR CLINICAL CANCER RESEARCH

Forskningsrådet

KREFTFORENINGEN

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High-throughput Chemical Biology Screening Platform
NCMM
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