Development of a high throughpuit in vitro screening platform to identify novel inducers of immunological cell death

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ICD, a non-conventional type of apoptosis is associated with the activation of an adaptive immune response against dead cell associated antigens. Anthracyclines exert immunostimulatory effects that rely on ICD. It is desirable to explore if other molecules can increase cancer cell immunogenicity and be attractive candidates for (combination) immunotherapy. Based on this knowledge, we developed a high throughput in vitro screening platform enabling the identification of compounds that induce ATP secretion, CRT exposure and HMGB1 release. We first tested this platform on our Lead-like set, unveiling several Nanocyclix molecules to render cell death immunogenic.

In vitro detection of ICD inducers

Step 1: Identify lowest toxic dose
- 3 cell lines: U-2 OS, MDA-MB-231 and Hela-1-6
- 5 doses: 0.1, 0.2, 0.25, 0.5 and 1 µM
- 72h incubation followed by assessment of cell viability (CellTiter Glo) using EnVision plate reader
- Assay format: 96-well plate
- Cut-off: >75% viability

Step 2: Identify compounds that result in secreted ATP
- 3 cell lines: U-2 OS, MDA-MB-231 and Hela-1-6
- 5 doses: highest concentration chosen from Step 1
- 72h incubation followed by evaluation of cell viability (CellTiter Glo) and secreted ATP (blue assay)
- Assay format: 96-well plate
- Cut-off: >2x secreted ATP with >75% viability

In vitro detection of ICD inducers

OCS142 treatment results in ICD release in 3 cell lines at non-toxic concentration.

Surface calreticulin detection: IF (ThermoFisher antibody)

Nucleus: Blue

Analysis System (PerkinElmer)

Immunogenic cell death and Nanocyclix

Single some-agent ICD inducers in cancer:

- Anthracyclines (Epothilones, Heat shock proteins, etc.)
- Bortezomib
- Cyclophosphamide
- Tumor necrosis factor (TNF)
- Metformin
- Fatigue management

Nanocyclix compound library: Nanocyclix is a proprietary medicinal chemistry technology based on the macrocyclization of small Lead-like molecules. This leads to lower MW kinase inhibitors with a unique binding mode and mode of action. The shape complementarity between the inhibitor and the active site of the kinase is believed to result in high potency and selectivity.

A lead-like set of 2318 compounds was selected to screen for novel ICD inducers.

Conclusions

Here, we describe a general strategy for the identification of ICD inducers within large chemical libraries.

We have validated the capability of our ICD screening platform by identifying OCS142, a compound that elicits an ICD response - secreted ATP, CRT exposure and HMGB1 release.

U-2 OS cells
- AT non-toxic doses, MTX and Dox treatment did not result in an increase in HMGB1 release.
- High concentrations of OCS142 lead to HMGB1 release.

OCS142 treatment results in HMGB1 release in 3 cell lines at non-toxic concentration.

Surface HMGB1 detection:

- HMGB1 is detectable after MTX and Dox treatment and can be used as an ICD read-out.

DAMPs (ATP, CRT, HSPs and HMGB1) released during immunogenic cell death (ICD) trigger and activate immune cells (DC, monocytes, T cells) to recognize tumor (neo)-antigens.

DMSO 0.2% 100% 100% 100% 100%

Dox 0.25 96% 429% Dox 0.5 98% 487%