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

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Introduction

Immunogenic cell death and Nanocyclix

Some single-agent ICD inducers in cancer:

<table>
<thead>
<tr>
<th>ICID inducer</th>
<th>Stage of cell death</th>
<th>Referent cell death pathway</th>
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</thead>
<tbody>
<tr>
<td>HMGB1</td>
<td>Necrosis</td>
<td>ATP, apoptosis/secretory and necrosis</td>
</tr>
<tr>
<td>Calreticulin</td>
<td>Mostly secreted</td>
<td>ICD, apoptosis/secretory and necrosis</td>
</tr>
<tr>
<td>Heat shock proteins (HSPs)</td>
<td>Surface exposure, active secretion or passive release</td>
<td>ICD, apoptosis/secretory and necrosis</td>
</tr>
<tr>
<td>High mobility group box 1 (HMGB1)</td>
<td>Mostly secreted; sometimes actively released</td>
<td>ICD, secondary necrosis, necrosis</td>
</tr>
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Nanocyclix compound library: Nanocyclix® is a proprietary medicinal chemistry technology based on the macrocyclization of small lead-like molecules. This led to small 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.

In vitro detection of ICD inducers - Strategy

Step 1: Identify lowest toxic dose

- 3 cell lines: U-2 OS (human), MDA-MB-231 (human) and Hepa 1-6 (mouse)
- 9 doses: 0.1, 0.5, 1.25, 2.5, 5, 10, 25, 50, 100 µM
- 72h incubation followed by assessment of cell viability (CellTiter-Blue) using EnVision well plate reader
- Assay format: 384-well plate
- Cut-off: >75% viability

Screening strategy for identification of HTS

144 hits

Step 2: Identify compounds that result in secreted ATP at non-toxic dose

- 3 cell lines: U-2 OS (human), MDA-MB-231 (human) and Hepa 1-6 (mouse)
- 5 doses: highest concentration chosen from Step 1
- 72h incubation followed by evaluation of cell viability (CellTiter-Blue) and secreted ATP (Cytosense)
- Assay format: 96-well plate
- Cut-off: >75% secreted ATP with >75% viability

24 hits

HMG1B release: ELISA (B. International)

ODS142 treatment results in an increase in secreted ATP at non-toxic concentration.

Surface HSP90: IF (alkaline antibody)

Surface HSP90 is detectable after MTX and Dox treatment and can be used as an ICD read-out.

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 ODS142, a compound that elicits an ICD response - secreted ATP, HMG1B release and surface CRT.