Real-Time Kinetic Data in the Successful Development of an Inhibition of Proliferation Potency Bioassay

Sophie Moncrieff[1], Eric Cruickshank[1], Amy Graham[1], Dion Gilmour[1], Lauren Gilfllan[1], Lewis Campbell, Adele Hannigan, Fiona Miller[1], Anke Hartug[2] & Andrew Baron[1]

[1]RoukenBio, RoukenBio Discovery Centre, Newhut Road, Motherwell, ML 1 3ST, UK;
[2]Tanvex BioPharma, USA, inc., 10421 Pasific Center Court, San Diego, CA 92121, USA

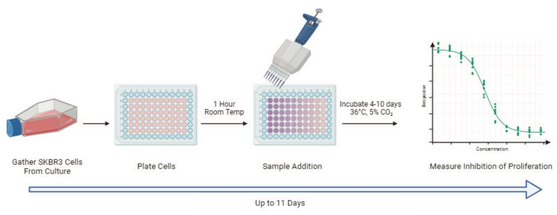
Introduction

The quantitative measurement of potency plays a critical role in biopharmaceutical development. Potency bioassays are essential tools for the measurement of potency but the complexity of measuring a meaningful biological response representative of the biotherapeutic's mechanism of action can pose significant challenges for assay development. Here we have applied a novel approach of utilizing the real-time kinetic capabilities of the xCELLigence to de-risk assay development for an inhibition of proliferation assay for an anti-HER2 monoclonal antibody.

Inhibition of proliferation assay principle and the potential risks for assay performance

Trastuzumab binds to the extracellular domain of HER2 and inhibits homodimerization, subsequently preventing HER2 mediated signaling responsible for proliferation in HER2 overexpressing cancer cells. SK-BR-3 cells over express HER2 and have been show to be susceptible to inhibition of proliferation by trastuzumab. SK-BR-3 are typically incubated with varying concentrations of trastuzumab for extended incubation periods (4 - 10 days) prior to the measurement of proliferation.

Typical SK-BR-3 inhibition of proliferation assay set up



Long incubation periods potentially expose the assay to plate effects and day-to-day variation

Mapping assay steps against potential risks for assay reliability and robustness identified the sample incubation duration as a key risk factor. With a doubling time of 2-3 days for SK-BR-3 cells, long incubation periods are necessary to achieve sufficient sensitivity for anti-proliferative effects which potentially also make the assay sensitive to unwanted day-to-day effects from common random variables such as FBS lot, operator, plate position or cell passage number. For this reason, initial development was performed using real-time kinetic proliferation data from the xCELLigence RTCA system to visualize the effect of assay conditions throughout the incubation duration and between days.

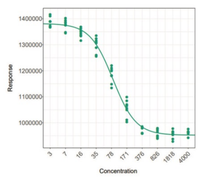
Transfer of assay parameters to a CMC-friendly assay format

xCELLigence allowed a data rich approach to understanding how assay conditions impact cellular responses and assay reliability however, practical considerations make it less well suited to routine use in a CMC setting. The final optimisation of the assay aimed to implement CellTitre-Glo® as an endpoint measure of proliferation, define the plate layout and replication approach as well as the statistical parameters.

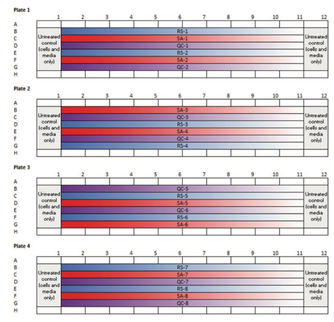
Further optimisation in a CMC-friendly format provided final assay parameters

Parameter	Optimisation
CellTiter-Glo Incubation Period	Evaluate 30-120 min. Defined 120 min as the optimal incubation period.
Use of FBS starvation	The use of 0% FBS starvation was not compatible with the assay plates used for CellTiter-Glo endpoint thus, the starvation period was omitted.
Trastuzumab concentration series optimisation	Final concentration series was 4000ng/mL with a 1 in 2.2 dilution step.
Plate map and replication approach	Analysis of plate effects and sample positioning bias over different numbers of plates and levels of sample replication was performed.

Table showing parameters investigated and results for final optimisation of the assay using a CellTiter-Glo® endpoint.



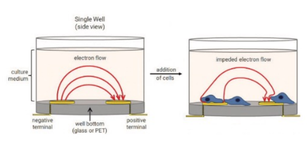
Representative 4-PL dose response curve following final optimisation. Response as luminescence units and concentration as ng/mL.



Final plate design using a blocking design for plate positional bias. Reference standard (RS), sample (SA) and QC reference standard (QC) as 8 replicates (4 independent x 2 technical replicates) over 4 plates.

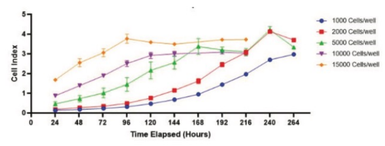
Application of real-time kinetic proliferation data in development

xCELLigence RTCA system principle



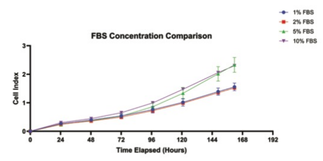
Microelectrodes fused to the bottom of a microtiter plate measure the presence of adherent cells which impede electron flow. Impedance is reported as cell index (CI) which increases as cells proliferate.

Kinetic growth profile of SK-BR-3 cells



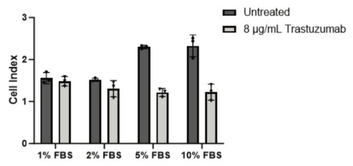
The data show cell seeding densities 2,000 - 5,000 cells/well at ~170 h achieved significant proliferation while remaining sub-confluent and thus, were appropriate conditions for the assay (n=3).

FBS concentrations of 5% and 10% avoid limiting proliferation in absence of trastuzumab



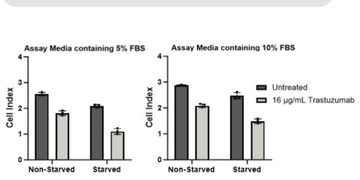
Data suggested that 5% and 10% FBS during assay incubation provide proliferative conditions for SK-BR-3 growth. Growth was limited with 1% and 2% FBS (n=3).

Higher FBS concentrations in assay media promote the anti-proliferative effect of trastuzumab



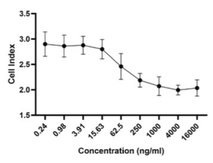
Data confirmed that 5% and 10% FBS during the sample incubation provided conditions most sensitive to anti-proliferative effects of trastuzumab (n=3).

Pre-assay 'starvation' of cells promote the anti-proliferative effects of trastuzumab.



SK-BR-3 cells were seeded at 3,000 cells per well in 'starvation media' containing 0% FBS or 'non-starved' media containing 5% or 10% FBS for 24 hours. Following starvation, cells were incubated in assay media (5% or 10% FBS) with or without 16 µg/mL trastuzumab for 6 days. Results showed the greatest anti-proliferative effect of trastuzumab with starvation (n=6).

Concentration dependent anti-proliferative effect of trastuzumab.



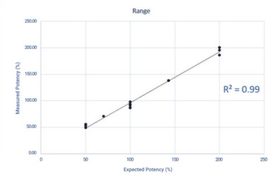
Dose response of SK-BR-3 cells treated with trastuzumab for 6 days following 24 hour 'starvation' with 0% FBS. Results were expressed as CI, error bars show StDev (n=6) with a single outlier removed at 250 ng/mL. Results showed a dose dependent anti-proliferative effect of trastuzumab.

Inhibition of proliferation assay qualification

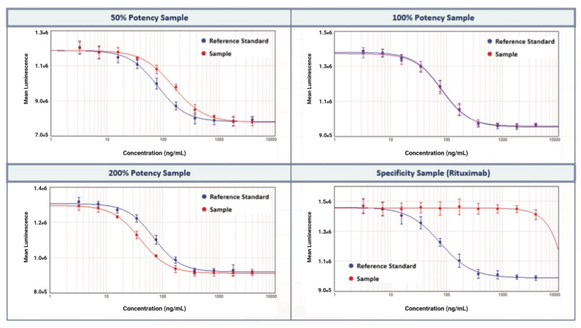
The assay was qualified for accuracy, precision, specificity and range using a range of sample concentrations relative to 100% reference standard. Three operators performed experiments over 11 days which incorporated different FBS lots and different cell passage numbers as typical sources of day-to-day variation.

Accurate and precise results were achieved over an assay range of 50 - 200%

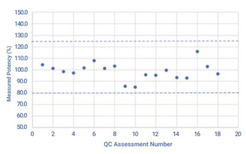
Expected Potency (%)	Measured Potency (%)	Accuracy (%)	Mean Accuracy (%)	%CV
50	55.8	111.6	105.4	6
	52.8	105.6		
	49.5	99.0		
70	70.8	101.1	101.1	n/a
100	87.3	87.3	91.3	5
	97.8	97.8		
	91.3	91.3		
	86.4	86.4		
	93.8	93.8		
143	138.5	96.9	96.9	n/a
200	186.3	93.2	97.1	4
	195.7	97.9		
	200.8	100.4		



Representative dose-response curves across the assay range



Highly consistent assay performance over time



QC relative potency was assessed in comparison to reference standard in each qualification run and demonstrated reliable performance of the method over time.

Groups	Count	Mean	%CV	P-value
>P+10	18	98.0	7.7	0.96
<P+10	13	98.2	7.0	

Groups	Count	Mean	%CV	P-value
Operator A	12	95.5	4.3	
Operator B	6	100.1	4.3	0.27
Operator C	13	99.6	9.7	

Groups	Count	Mean	%CV	P-value
FBS Lot 1	17	99.1	5.9	0.39
FBS Lot 2	14	96.9	8.8	

Performance of the assay was unaffected by different SK-BR-3 passage numbers, different operators or different FBS lots. Assessed by t-test or ANOVA, $p \geq 0.05$.

Summary

We have demonstrated the applicability of real-time kinetic data in the development of an anti-proliferation potency bioassay which provided a detailed understanding of the effects of assay conditions on cellular responses to produce a reliable bioassay with consistent performance. This understanding is important for the development of proliferation bioassays for process development, characterization, stability and biosimilar similarity assessments.

Scan the code

Download the digital version of this research poster and take all the latest advancements and data home in your pocket.