Development of a Neutralizing Anti-drug Antibody Detection Assay targeting the PD-1 Checkpoint Inhibitor, *Nivolumab* using a PD-1 /PD-L1 Blockade Bioassay

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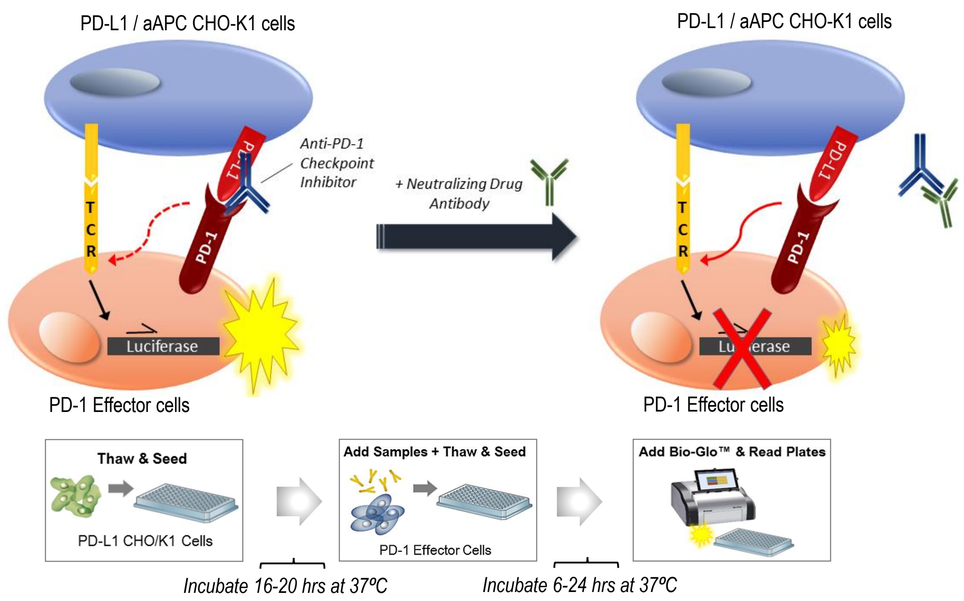
1 ABSTRACT

Nivolumab is a therapeutic monoclonal antibody (mAb) that targets programmed cell death 1 receptor (PD-1) and reverses T-cell exhaustion by inhibiting PD-1/PD-L1 interactions within the tumor microenvironment. It is one of many therapies utilizing the immune system checkpoint balance in order to activate the immune system and destroy cancer cells. As with any mAb, dosing with nivolumab can lead to the production of anti-drug antibodies, (ADA), which can exhibit neutralizing activity (NAbs). The objective of this study was to develop a neutralizing anti-nivolumab antibody screening assay using the PD-1/PD-L1 Blockade Bioassay (Promega). This bioassay is primarily designed to measure the potency and stability of mAbs and other biologics known to inhibit the PD-1/PD-L1 interaction. Here we present the development of a functional cell-based NAb assay using the PD-1/PD-L1 Blockade Bioassay for the analysis of clinical serum samples. Results from the initial assessment of nivolumab activity for the NAb assay development were consistent with the previously developed potency assay, suggesting it is a highly reproducible cell-based format. Results for nivolumab activity for both the potency and NAb assay are presented, including critical NAb validation parameters: precision, selectivity, sensitivity and drug tolerance. Such optimized potency bioassays for checkpoint inhibitors have the potential to be developed into clinically relevant NAb assays with reduced variability and can be used to easily overcome common drawbacks associated with a cell-based format.

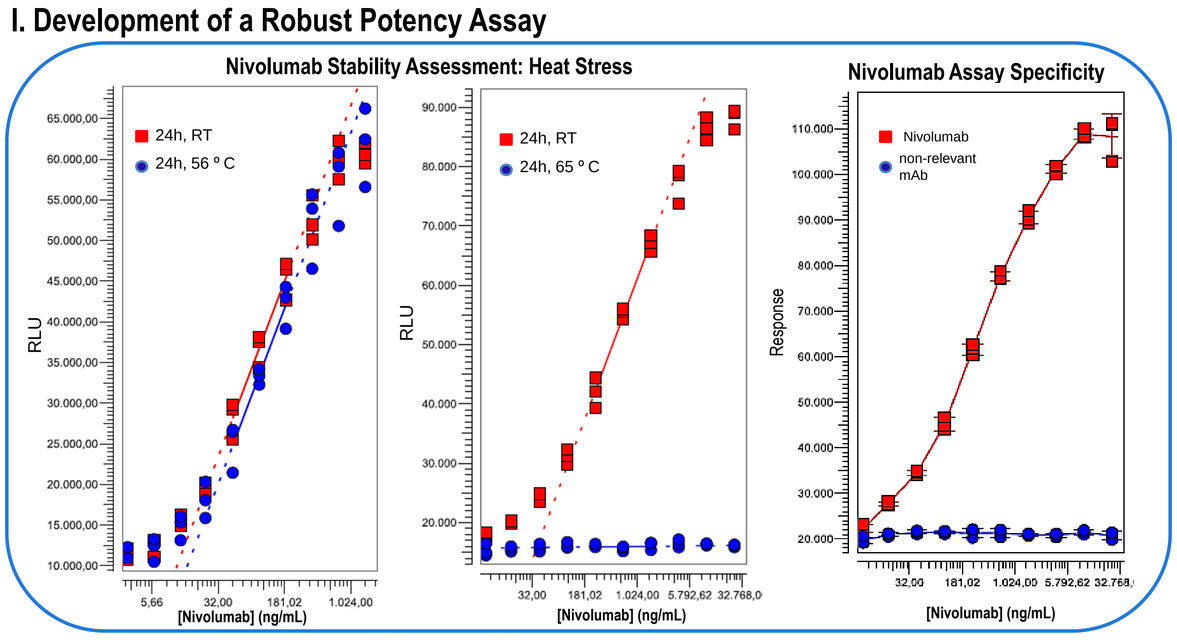
2 METHODS

The potency and NAb assay for nivolumab were carried out using the PD-1/PD-L1 Blockade Bioassay kit (by Promega). The kit uses two freeze and thaw cell lines; APC-mimicking cells expressing the PD-L1 ligand and a small TCR-activating protein and PD-1 Effector cells that expresses the PD-1 receptor and a T-cell receptor. In the presence of a PD-1/ PD-L1 checkpoint inhibitor, a T-cell receptor pathway is activated resulting in a luminescent signal. Neutralizing anti-PD-1/ PDL-1 antibodies are measured by a reduction in luminescence following lowered drug availability for PD-1 inhibition.



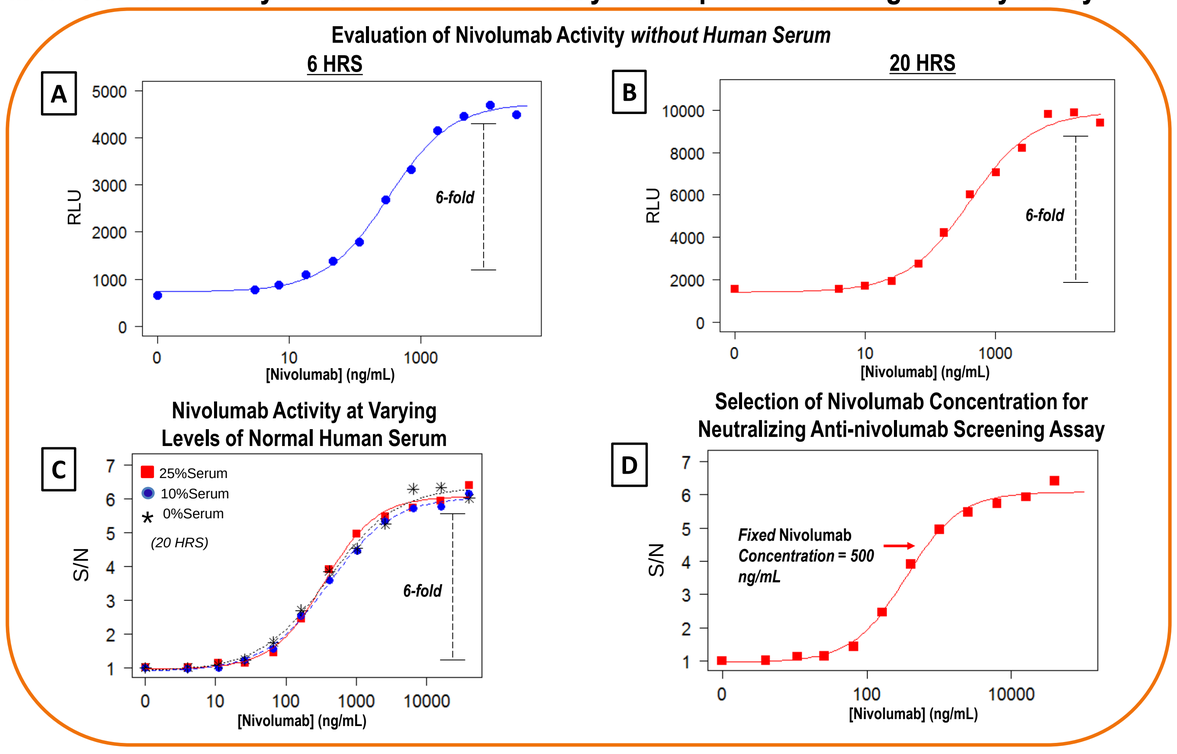
3 RESULTS

I. Development of a Robust Potency Assay



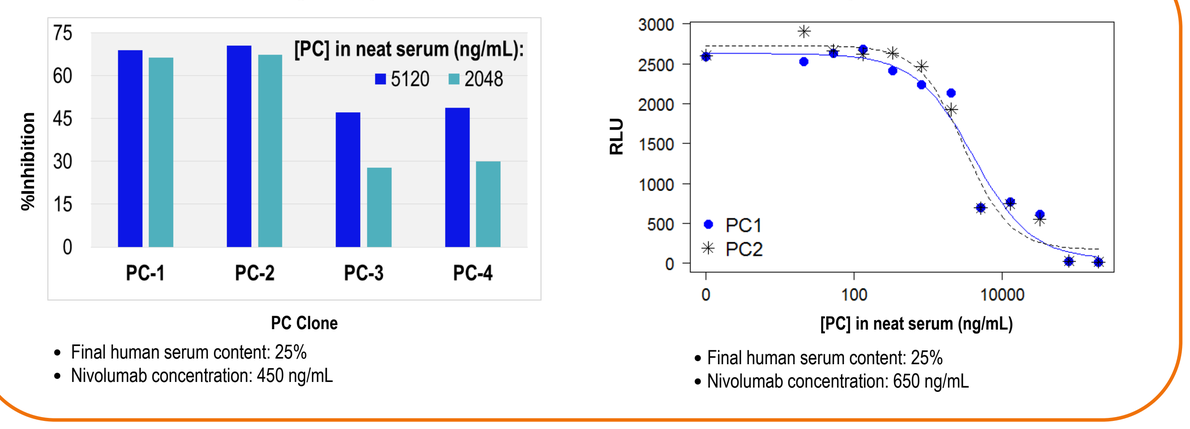
Assay tolerant to heat stress up to 56 ºC and specificity for nivolumab vs. a non-relevant monoclonal Antibody using the PD-1/PD-L1 Blockade Bioassay.

II. Nivolumab Activity Evaluation for NAb Assay Development following Potency Assay



The PD-1/PD-L1 Blockade Bioassay demonstrated similar drug activity between incubation times (A, B). No significant difference was found between the different %matrix tested (C). A fixed nivolumab concentration was selected from the dose-response curve at 20 HRS (D).

III. Assessment of Positive Anti-Nivolumab Control



- Final human serum content: 25%
- Nivolumab concentration: 450 ng/mL

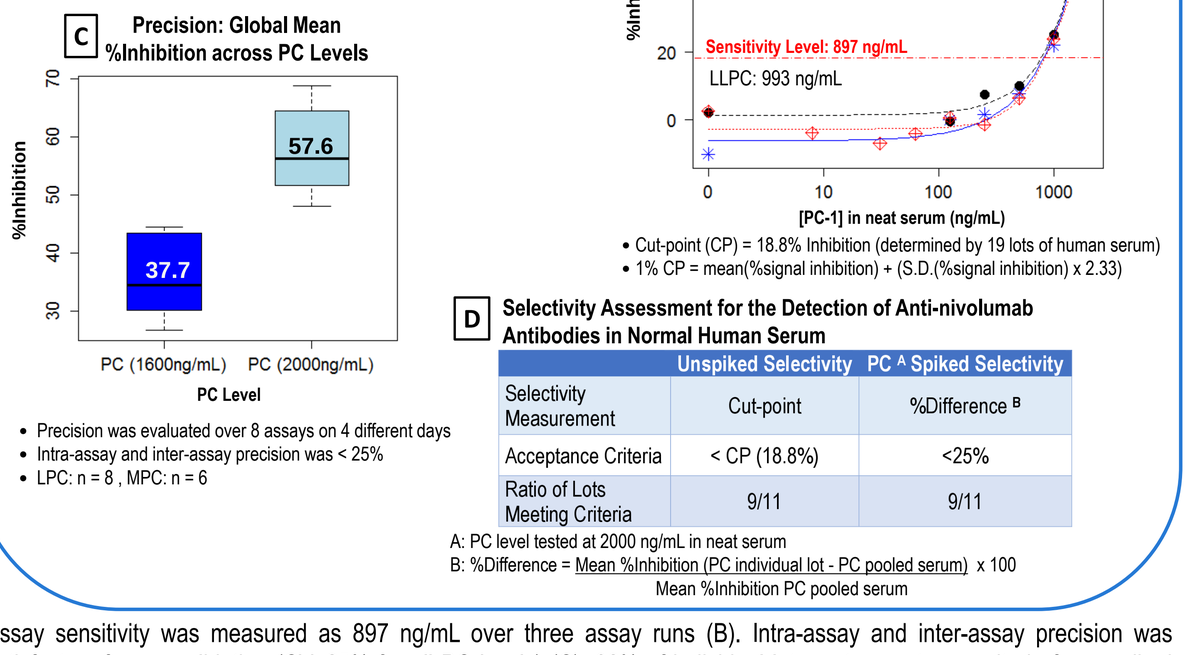
- Final human serum content: 25%
- Nivolumab concentration: 650 ng/mL

NAb activity was assessed for four humanized, monoclonal anti-nivolumab antibody clones to select a suitable neutralizing positive assay control. PC-1 and PC-2 were noticeably more potent inhibitors than PC-3 and PC-4. PC-1 was selected as the positive assay control.

IV. Pre-Validation Assessment for NAb Assay targeting Nivolumab

A

Pre-validation Parameters Evaluated	
Cut-point *(data not shown)*	Sensitivity
Intra- & Inter- assay Precision	Selectivity
	Drug Tolerance *(data not shown)*



- Precision was evaluated over 8 assays on 4 different days
- Intra-assay and inter-assay precision was < 25%
- LPC: n = 8 , MPC: n = 6

- Cut-point (CP) = 18.8% Inhibition (determined by 19 lots of human serum)
- 1% CP = mean(%signal inhibition) + (S.D.(%signal inhibition) x 2.33)

D **Selectivity Assessment for the Detection of Anti-nivolumab Antibodies in Normal Human Serum**

	Unspiked Selectivity	PC [A] Spiked Selectivity
Selectivity Measurement	Cut-point	%Difference [B]
Acceptance Criteria	< CP (18.8%)	<25%
Ratio of Lots Meeting Criteria	9/11	9/11

A: PC level tested at 2000 ng/mL in neat serum

B: $\%\text{Difference} = \frac{\text{Mean \%Inhibition (PC individual lot - PC pooled serum)}}{\text{Mean \%Inhibition PC pooled serum}} \times 100$

Assay sensitivity was measured as 897 ng/mL over three assay runs (B). Intra-assay and inter-assay precision was satisfactory for pre-validation (CV<25% for all PC levels) (C). 82% of individual lots met acceptance criteria for unspiked and spiked selectivity (D).

4 CONCLUSION

- NAb assays rely on an extensive developmental process often based on potency assays. Such pre-optimized platforms greatly improve the lifecycle development of immunogenicity testing.
- The time allocated to developing a NAb assay was greatly reduced following a potency assay using the PD-1 /PD-L1 Blockade Bioassay targeting nivolumab (8 occasions including pre-validation parameters).
- Testing of key validation parameters revealed a sufficiently sensitive and precise NAb assay with considerable serum tolerance.
- Drug tolerance was assessed at a PC level of 1600 ng/mL. The current assay format is tolerant up to 394 ng/mL and will likely require an additional pre-treatment step.
- Assay is suitable as a platform assay to assess NAbs in serum samples as well as for the determination of biological activity in drug substance and drug product for lot-release and stability testing purposes.