SARTORIUS

Application Note

November 12, 2019

Keywords or phrases:
Biosimilar, Nivolumab, IgG, IgG4, Monoclonal Antibody (mAb), SPR, Lack of effector function, Affinity

Nivolumab (Opdivo®)

SPR Assays to the Assess Similarity Between Innovator and Biosimilar Versions of Nivolumab (Opdivo®)

Daniel O'Loughlin
Sartorius Stedim BioOutsource Ltd., 1 Technology Terrace, Todd Campus, West of Scotland Science Park, Glasgow, G20 0XA, Scotland

Correspondence
E-Mail: info@sartorius.com

Abstract

Nivolumab (Opdivo®) is a human monoclonal antibody (IgG4 isotype) that binds to the protein PD-1. Nivolumab works as a checkpoint inhibitor, blocking the interaction between PD-1 and its ligands (PD-L1 and PD-L2), which would normally lead to the inhibition of T cell activation. Nivolumab was first approved for the treatment of melanoma in 2014, and, since then, the drug has also been approved for use in the treatment of other advanced cancers.
Surface Plasmon Resonance (SPR) is a label-free technique that can be used to probe molecular interactions in real-time, yielding information on interaction kinetics, binding affinity, and binding responses. Sartorius has developed a suite of SPR assays for evaluating the similarity of biosimilar versions of nivolumab using the Biacore T200 series of SPR instruments. Assays have been developed for the following interactions of nivolumab:

- Fc Gamma Receptors
  - **FcγRI (CD64)***
  - **FcγRIIa (CD32a – including H variant)***
  - **FcγRIIb (CD32b)***
  - FcγRIIIa (CD16a)
  - FcγRIIIb (CD16b)
- **FcRn (Neonatal Receptor)***
- PD-1 (Target Binding)

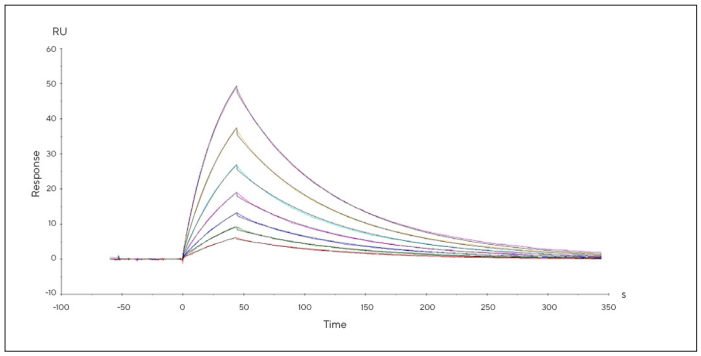
* Assays that have been fully qualified with respect to relative affinity and relative binding.

The PD-1 target binding assay has accuracy data available and can be qualified for a client's specific product. Both the FcγRIIIa and FcγIIIb demonstrate the lack of nivolumab binding and therefore cannot be qualified. Example data and a schematic for a selection of the receptors is shown in the following passage.

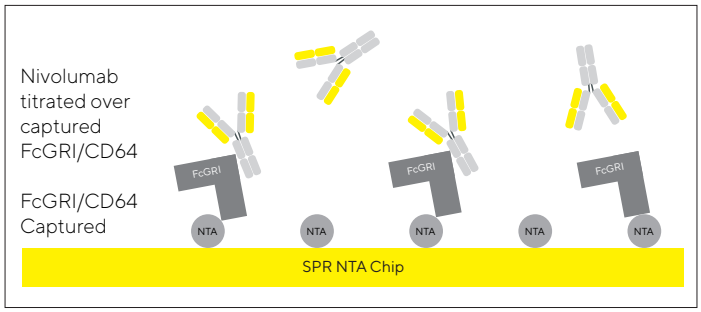
FcγRI (CD64)

IgG4 antibodies bind to FcγRI with a lower affinity than IgG1 antibodies[1]. Sartorius has developed a capture approach using a 1:1 kinetic fit with good quality attributes (low Chi2 value) as well as precise binding data. This assay has been qualified in the range 50 to 200 percent for relative binding and 70 to 143 percent for relative affinity.

FcγRI – 1:1 Kinetic Fit



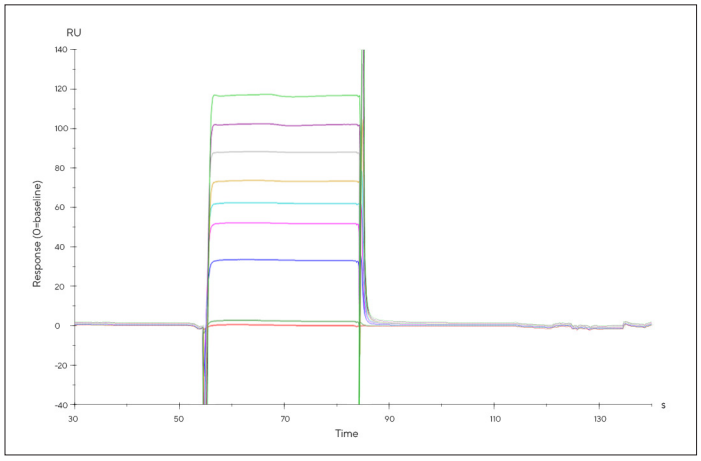
Schematic of assay set up



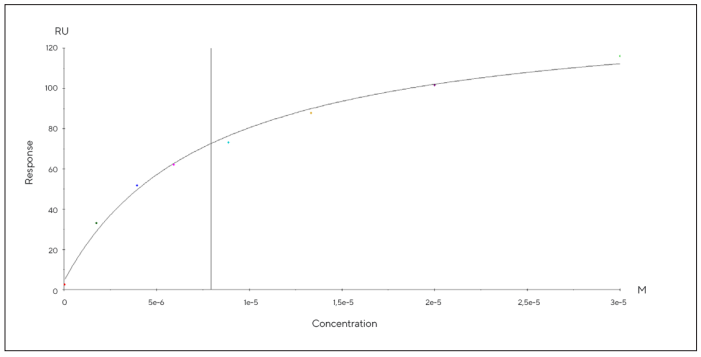
FcγRIIa (CD32a – including H genotype)

IgG4 antibodies also demonstrate weaker binding to FcγRIIa than IgG1 antibodies[1]. Following an immobilization, approach we demonstrate steady-state affinity fits with good quality attributes (low Chi2 value) as well as precise data. FcγIIa R genotype data is presented below, the assay has been qualified in the range of 50 to 200 percent for both relative binding and relative affinity.

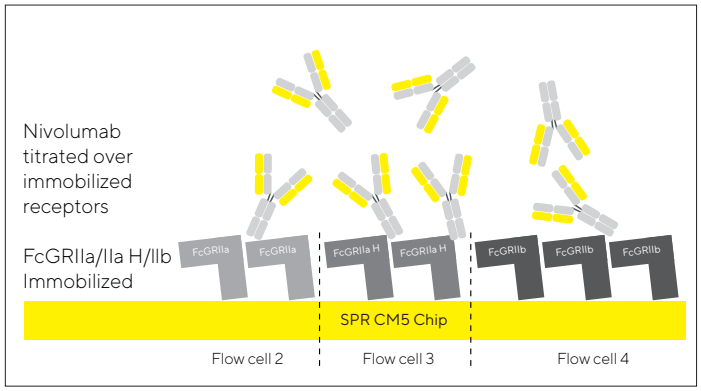
FcγRIIa R variant – Raw Sensorgrams



FcγRIIa R variant – Steady-State Affinity Fit



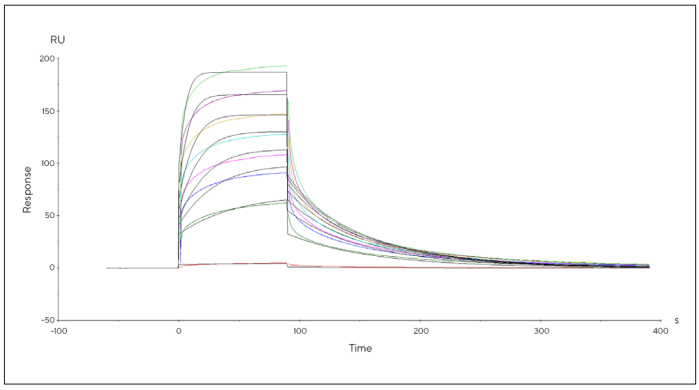
Schematic of assay set up



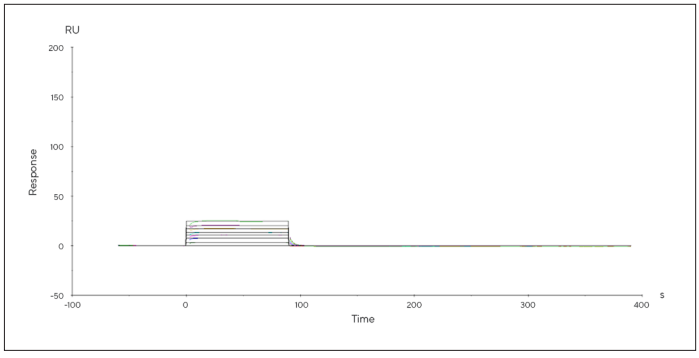
FcγRIIIa (CD16a– including F genotype variant)

IgG4 antibodies demonstrate far weaker binding to FcγRIIIa than IgG1 antibodies[1]. Sartorius has developed a direct immobilization approach providing, as anticipated, workable binding data but poor kinetic/steady-state fit data for the FcγIIIa V genotype. As a positive control, an IgG1 was assessed alongside nivolumab in the same assay. Nivolumab demonstrates very fast on/off rates and a significantly reduced signal compared to the IgG1.

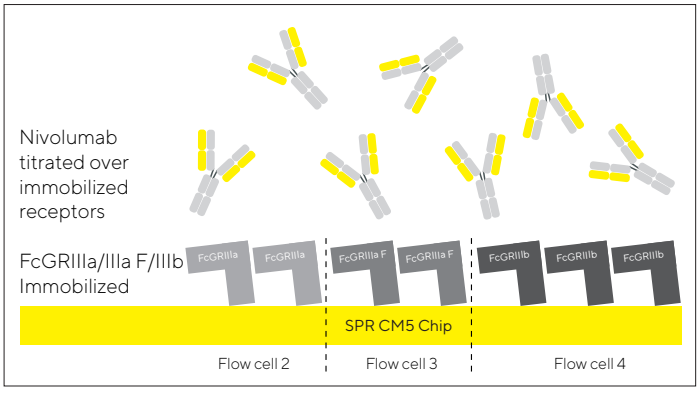
IgG1
FcγRIIIa V variant – 1:1 Kinetic Fit



IgG4 (nivolumab)
FcγRIIIa V variant – 1:1 Kinetic Fit



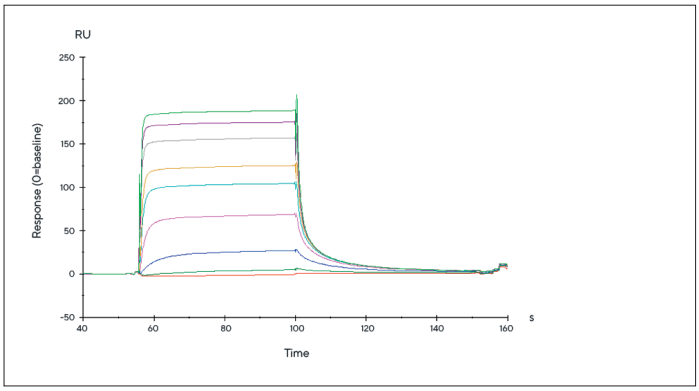
Schematic of assay set up



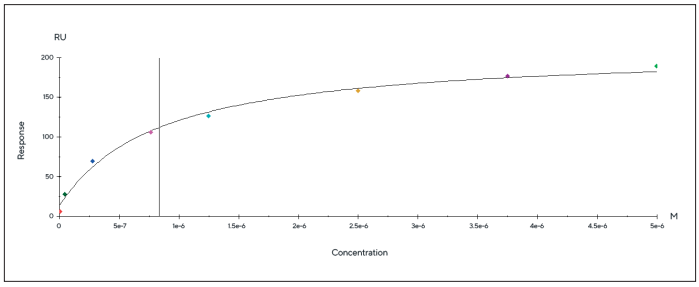
FcRn (Neonatal Fc Receptor)

FcRn rescues antibodies from lysosomal destruction within endothelial cells by binding in a pH dependent manner, increasing the half-life of the therapeutic. We have developed a capture-based approach to characterize the binding of biosimilar and innovator nivolumab binding FcRn. This assay has been qualified in the range of 50 to 200 percent for relative binding and relative affinity. Data below demonstrate the raw sensorgrams for FcRn binding as well as steady-state fits.

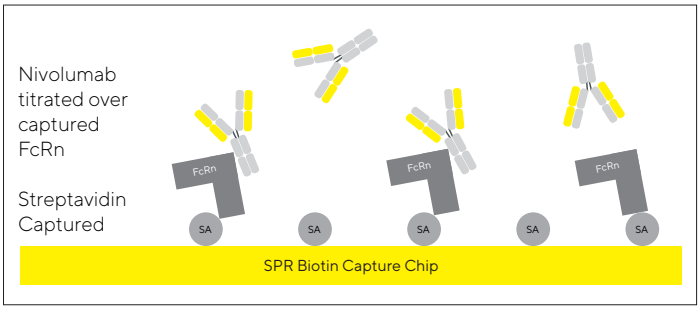
FcRn – Raw Sensorgrams



FcRn – Steady-State Affinity



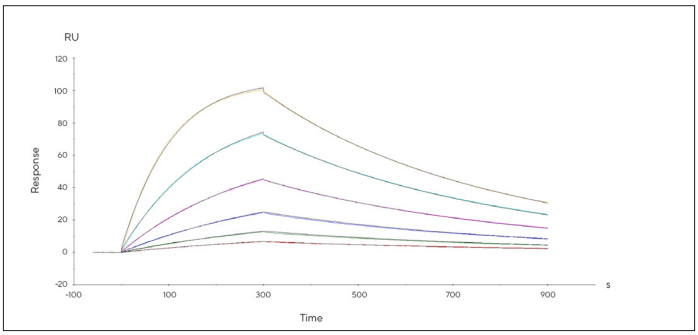
Schematic of assay set up



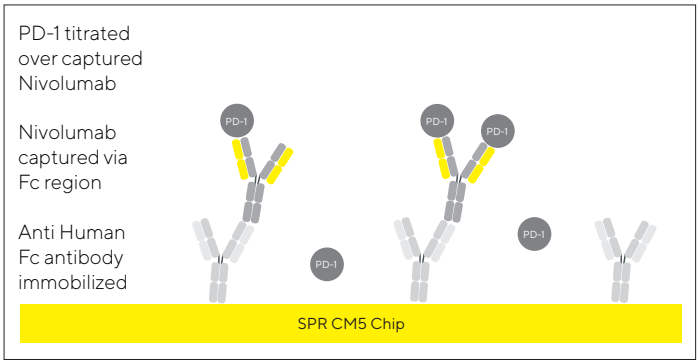
PD-1 (Target binding)

Target binding is a critical function of therapeutic antibodies and is fundamental for their efficacy. Sartorius has developed a capture-based assay to compare the binding to PD-1 of biosimilars with innovator batches. In preliminary accuracy assessments, we have shown that the assay is accurate for relative affinity and relative binding in the range of 50 to 200 percent. An example 1:1 kinetic fit (low Chi2 values and precise affinity are observed) is shown below.

PD-1 – 1:1 Kinetic Fit



Schematic of assay set up



Conclusion

The success of Opdivo® has highlighted nivolumab as a target for biosimilar developers around the world. Sartorius has developed and qualified SPR assays to characterize the binding and affinity of biosimilar and innovator nivolumab for all seven Fc gamma receptors, FcRn and PD-1. These assays will also represent a platform approach for characterization of other anti-PD-1 mAbs and IgG4 biosimilars and innovators. The SPR assays compliment our ELISAs and Bioassays for nivolumab characterization, allowing a complete package of data to be assembled demonstrating the similarity of biosimilar to innovator using an orthogonal approach.

References

[1]Specificity and affinity of human Fc gamma receptors and their polymorphic variants for human IgG subclasses. Bruhns et al, Blood. 2009 Apr 16; 113(16):3716-25

Germany
Sartorius Stedim Biotech GmbH
August-Spindler-Straße 11
37079 Göttingen
Phone +49 551 308 0

USA
Sartorius Stedim North America Inc.
565 Johnson Avenue
Bohemia, NY 11716
Toll-Free +1 800 368 7178

For further contacts, visit
www.sartorius.com