Novel Small-molecule Antagonists of the PD-1/PD-L1 Axis That Mediate Cell Surface PD-L1 Dimerization and Internalization

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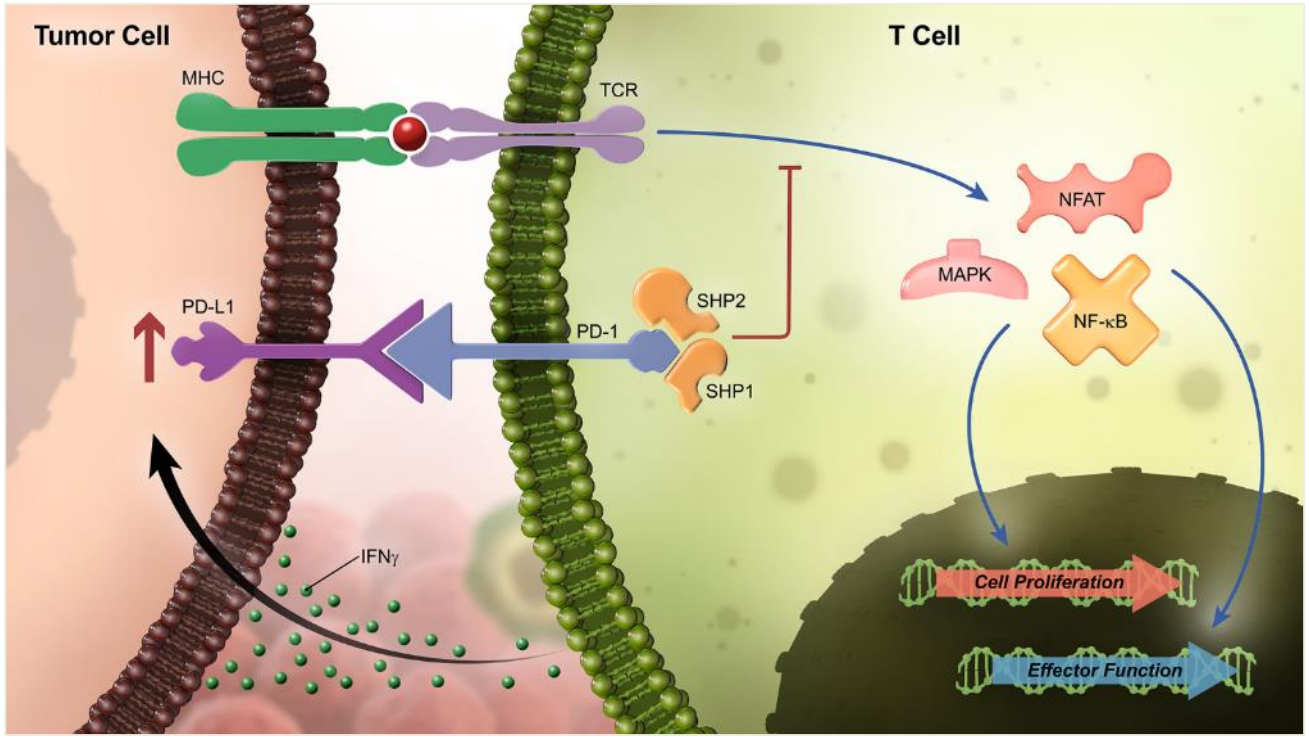
Abstract: 4483. Presented AACR Annual Meeting, April 2, 2019, Atlanta, GA

Disclosures

- All authors are current or previous employees of Incyte, and own stock of Incyte

Checkpoint Proteins PD-1 and PD-L1 Suppress Activated T-cell Signaling



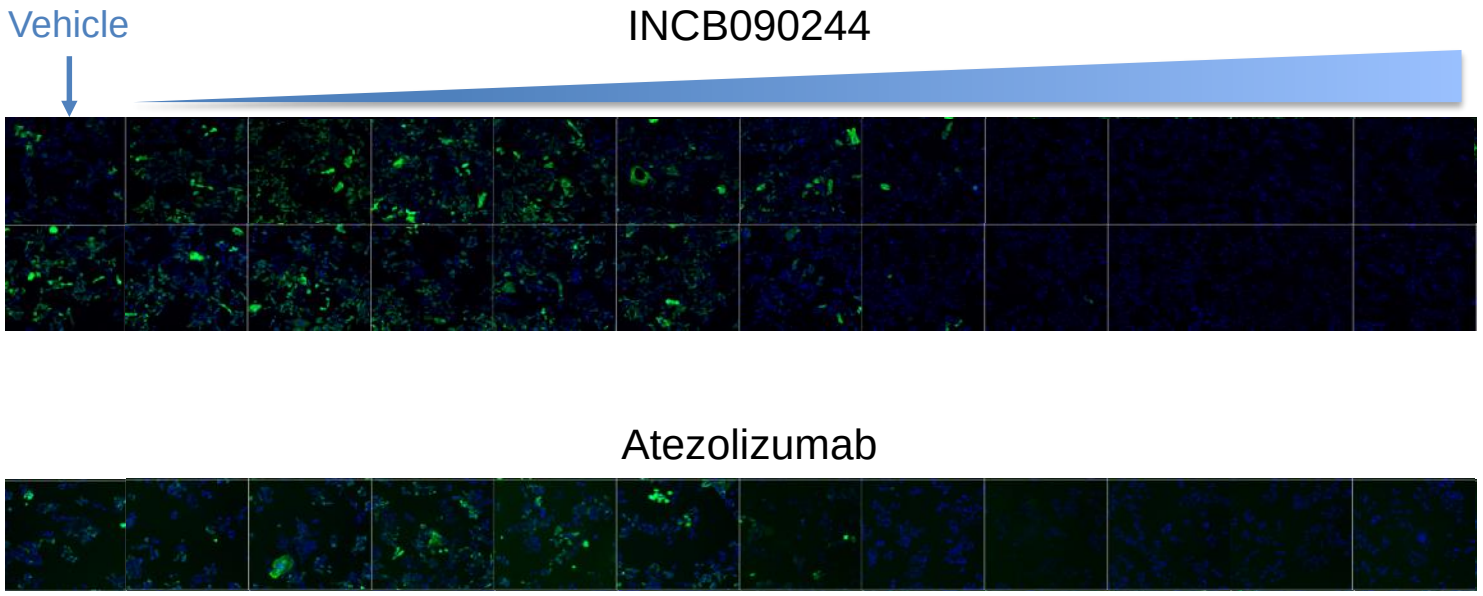
- Blocking PD-1/PD-L1 interaction is effective in reversing immune suppression by tumor cells[1,2]
- Approved therapies against PD-1 or PD-L1 are monoclonal antibodies[1,2]
- Small-molecule inhibitors may offer advantages
  - Oral dosing
  - Small-molecule combinations
  - Titratable control of drug levels
  - Improved tumor penetration

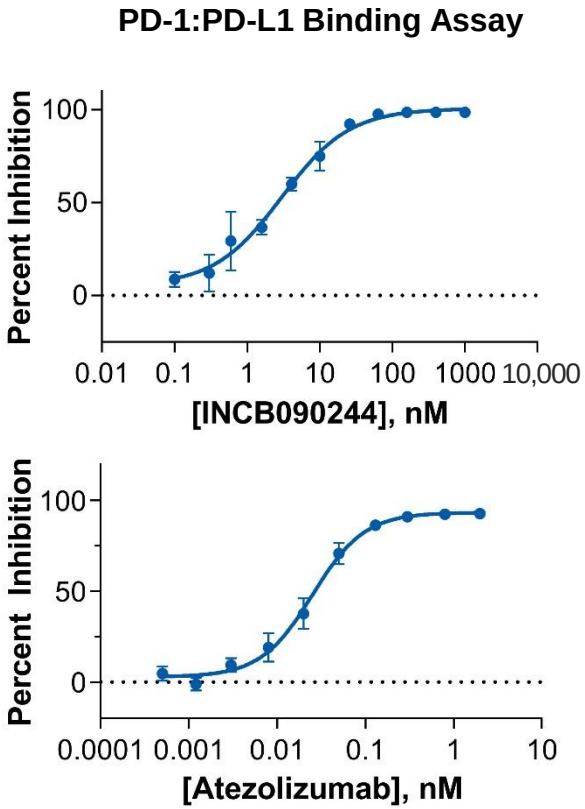
Objective: **To discover small-molecule inhibitors of PD-1/PD-L1 interaction with drug-like properties and activity equivalent to therapeutic antibodies**

1. Ribas A and Wolchok JD. *Science.* 2018;359:1350−55. **2.** Sharpe AH and Pauken KE. *Nat Rev Immunol.* 2018;18:153−67.

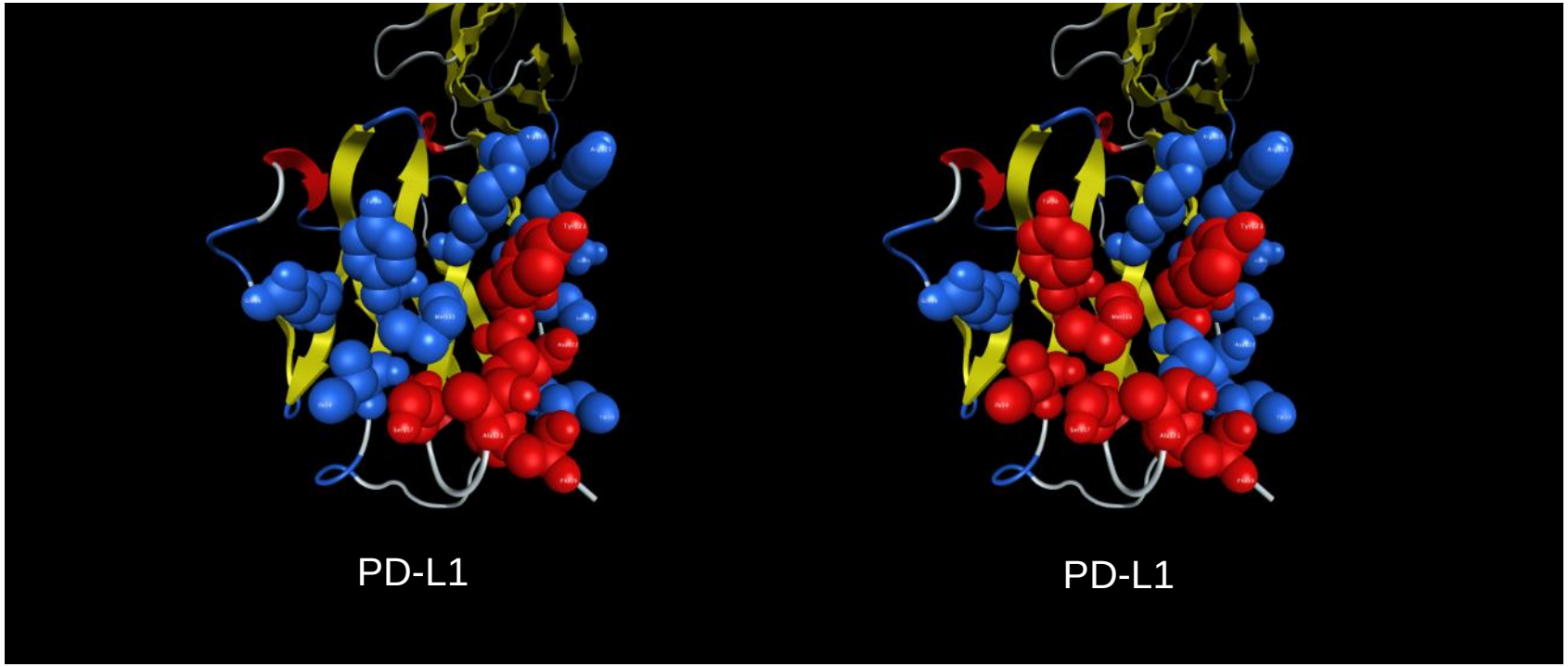
Small-molecule Inhibitors Were Identified That Potently Inhibited PD-1 Binding to PD-L1

- Activity of inhibitors phenocopied antibodies in biochemical assays but showed differentiated cellular MoA
- Lead inhibitor (INCB086550) has progressed into phase 1 clinical trials (NCT03762447)
- INCB090244 blocks PD-1/PD-L1 interactions in vitro and on cells





Small Molecules Block Sites of Interaction Between PD-1 and PD-L1



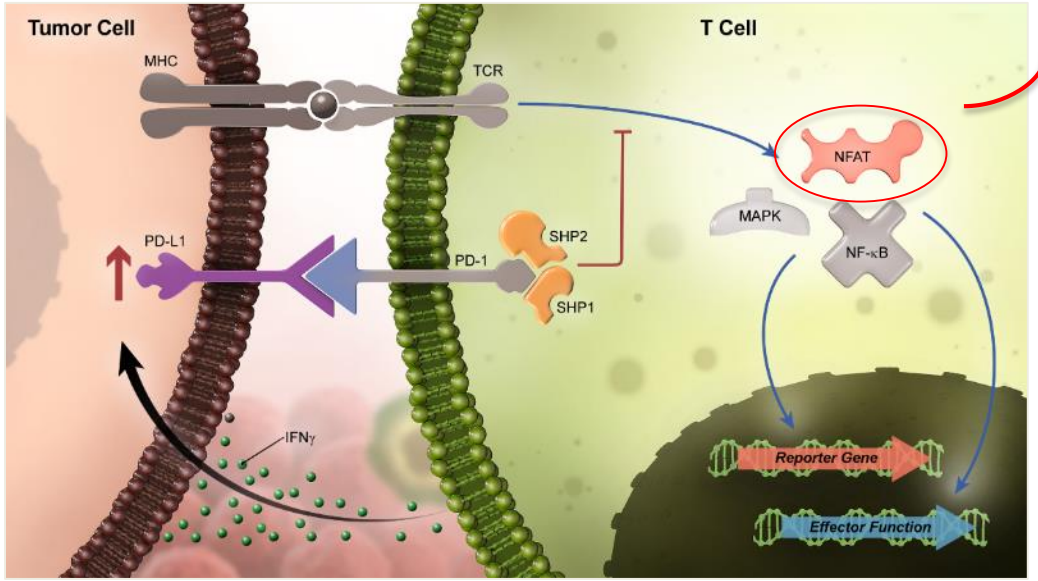
PD-1 binding contacts

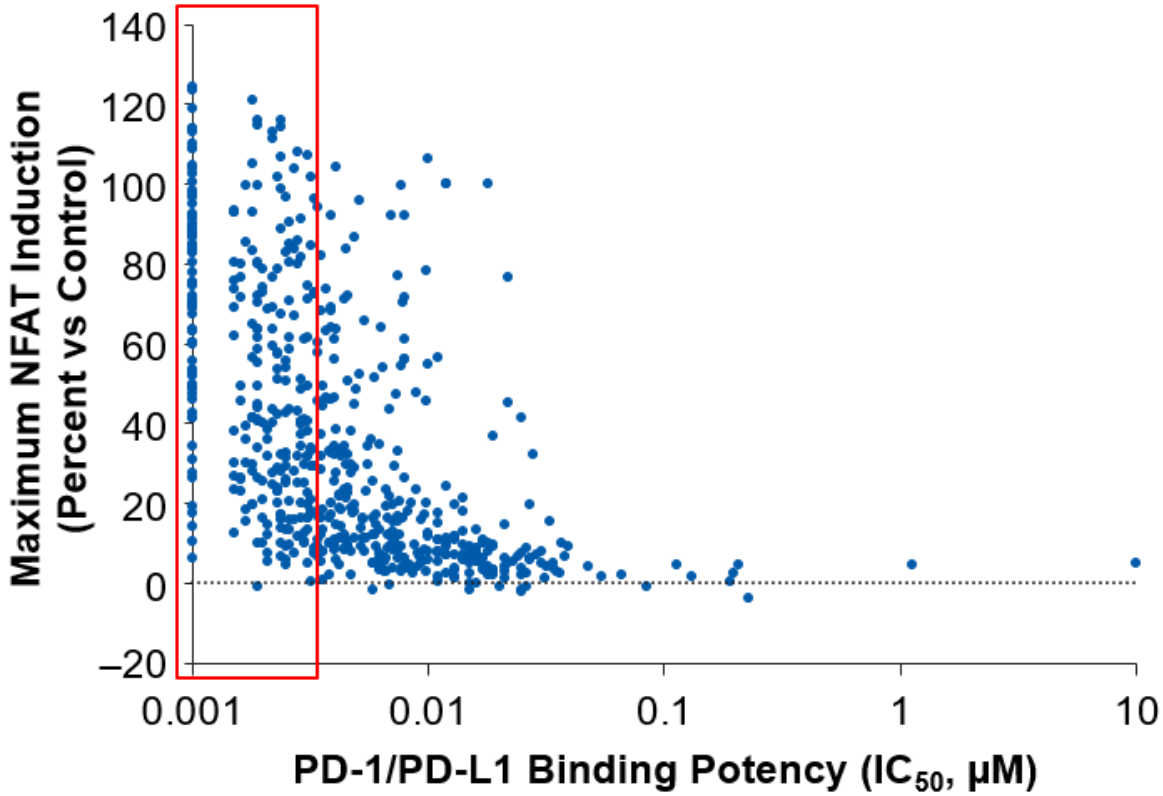
Small-molecule inhibitor contacts

- Mutagenesis experiments show residues on PD-L1 that are critical for binding to PD-1 or the inhibitor
  - Red: >5× shift in potency
  - Blue: <5× shift in potency
- Partially overlapping residues indicate competitive binding – confirmed by SPR and biochemical assays

Zak KM, et al. *Structure* 2015;23:2341−48.

Biochemical Potency did not Correlate With Cellular Activity in the NFAT Assay

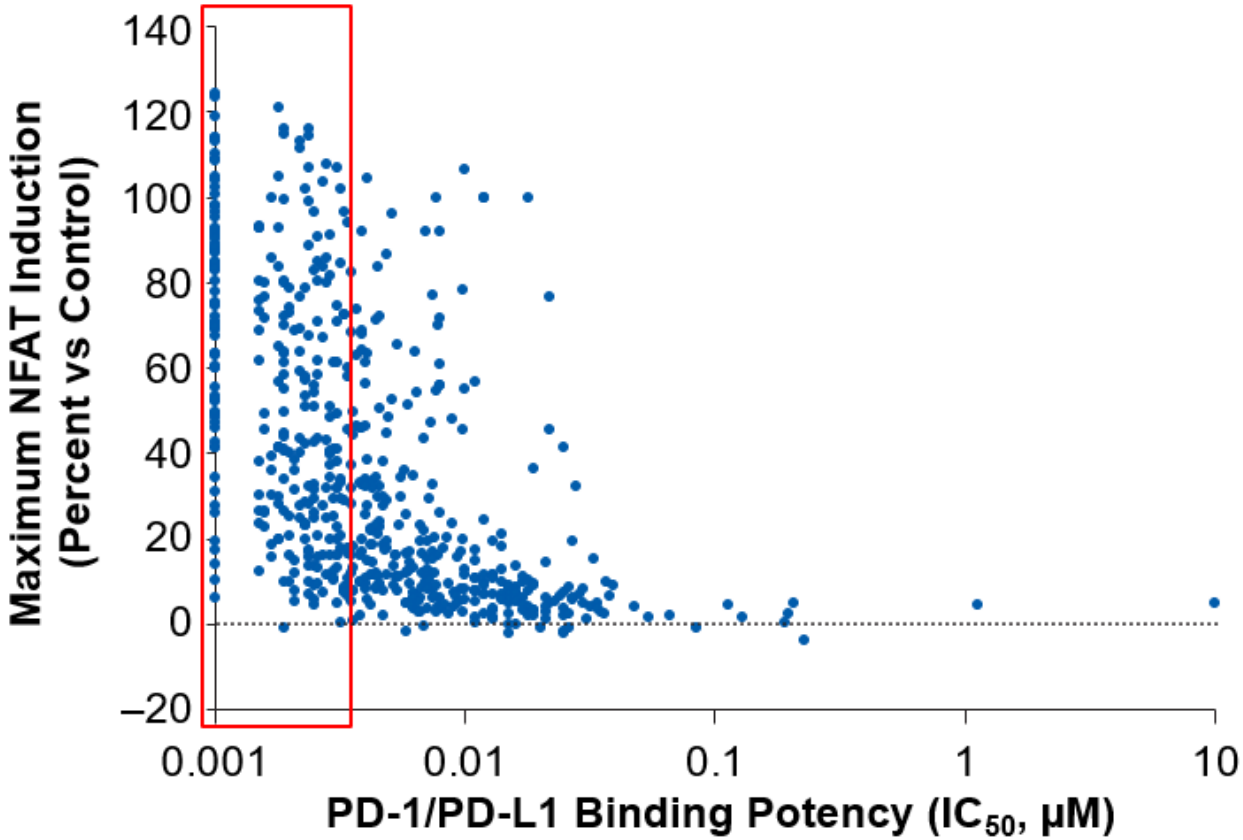




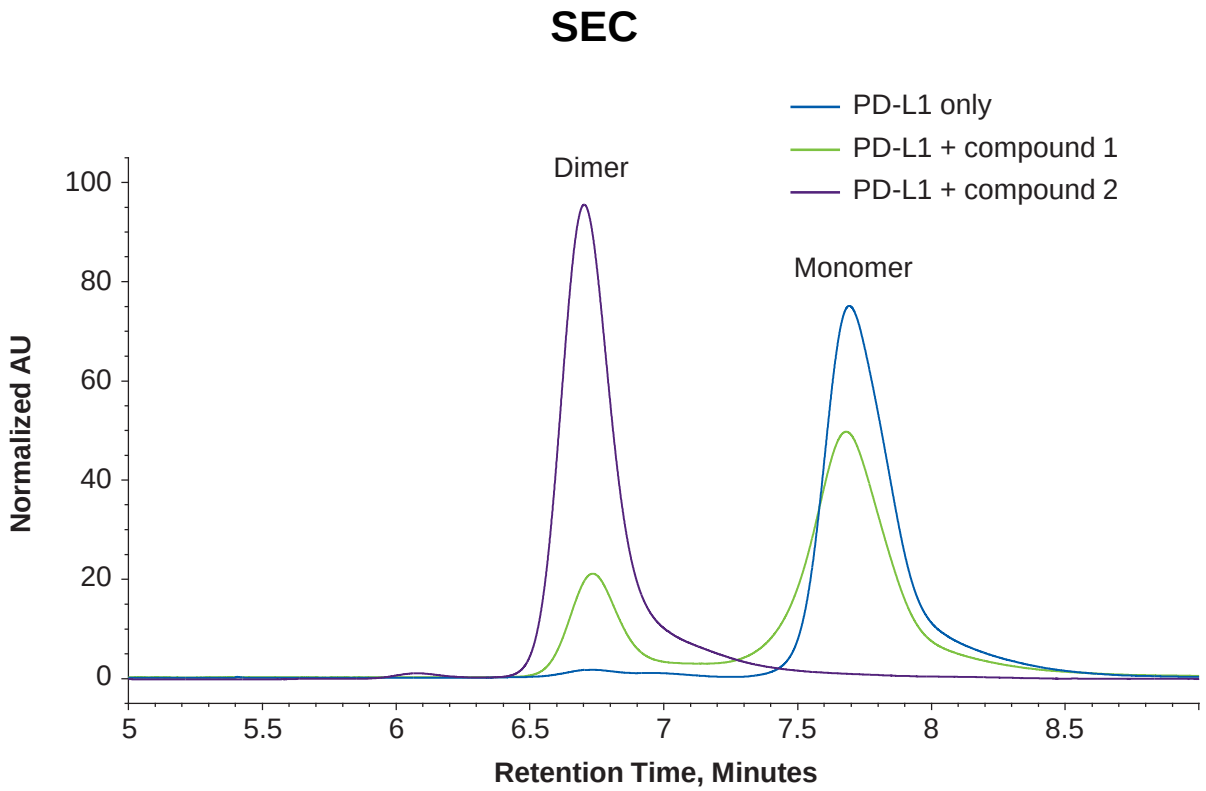
- SAR conundrum: compounds that were potent inhibitors had variable responses in functional cell assay
- Compounds could be classified into subsets based on activity in the various cellular readouts

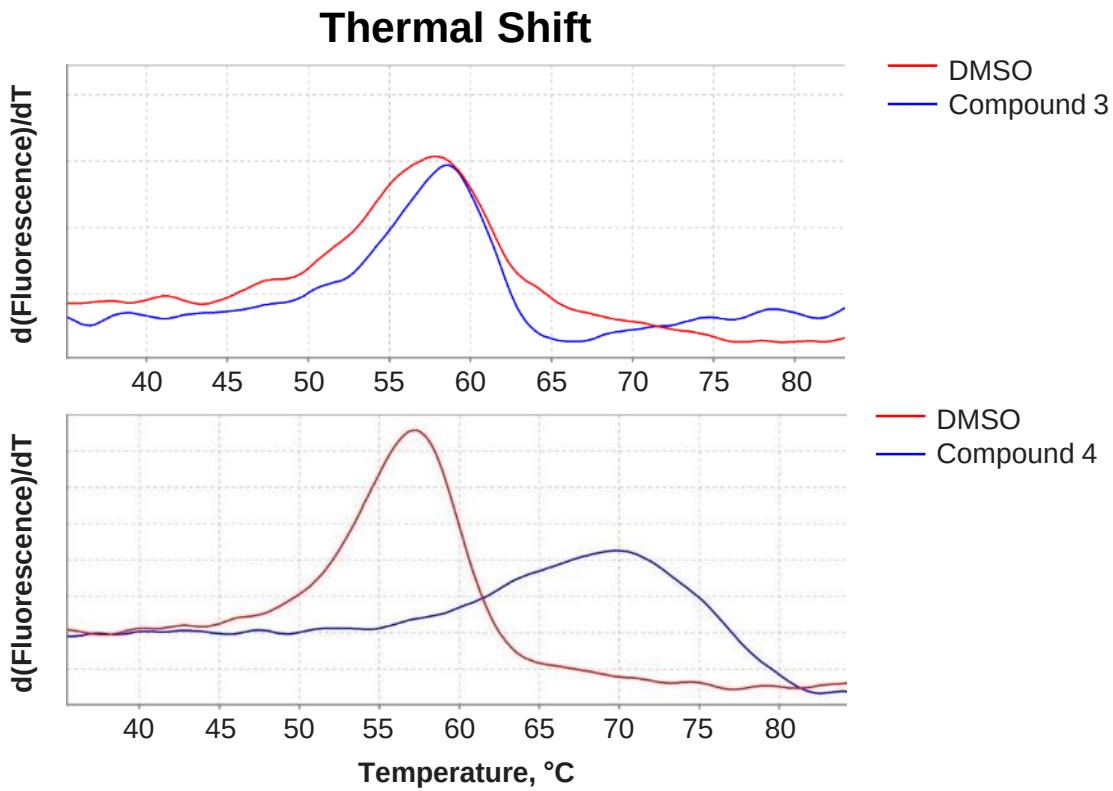
Understanding Mechanistic Differences Among Inhibitors

- Biochemical/biophysical
  - Size exclusion chromatography (SEC)
  - Thermal shift
  - PD-L1 dimerization FRET
- Cellular
  - NFAT reporter
  - Cellular internalization
  - T cell assays



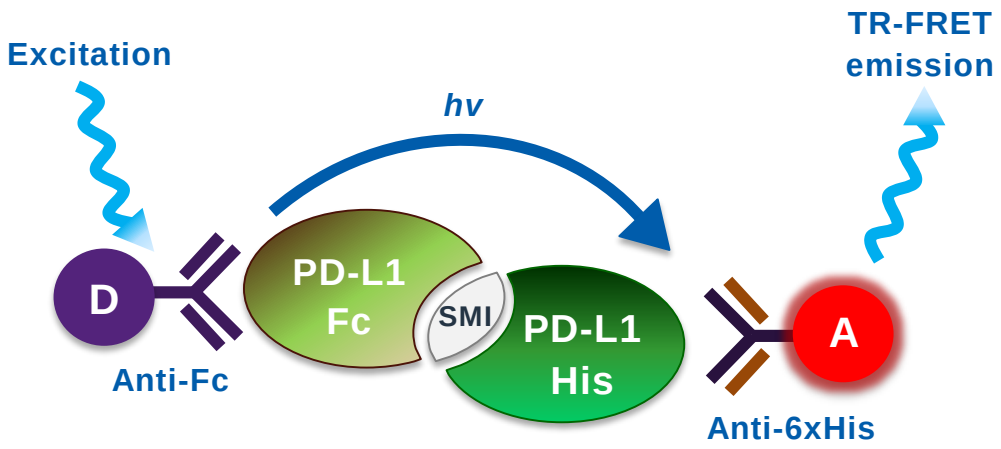
Biophysical Analyses Revealed Compound-induced Dimerization and Stabilization of PD-L1



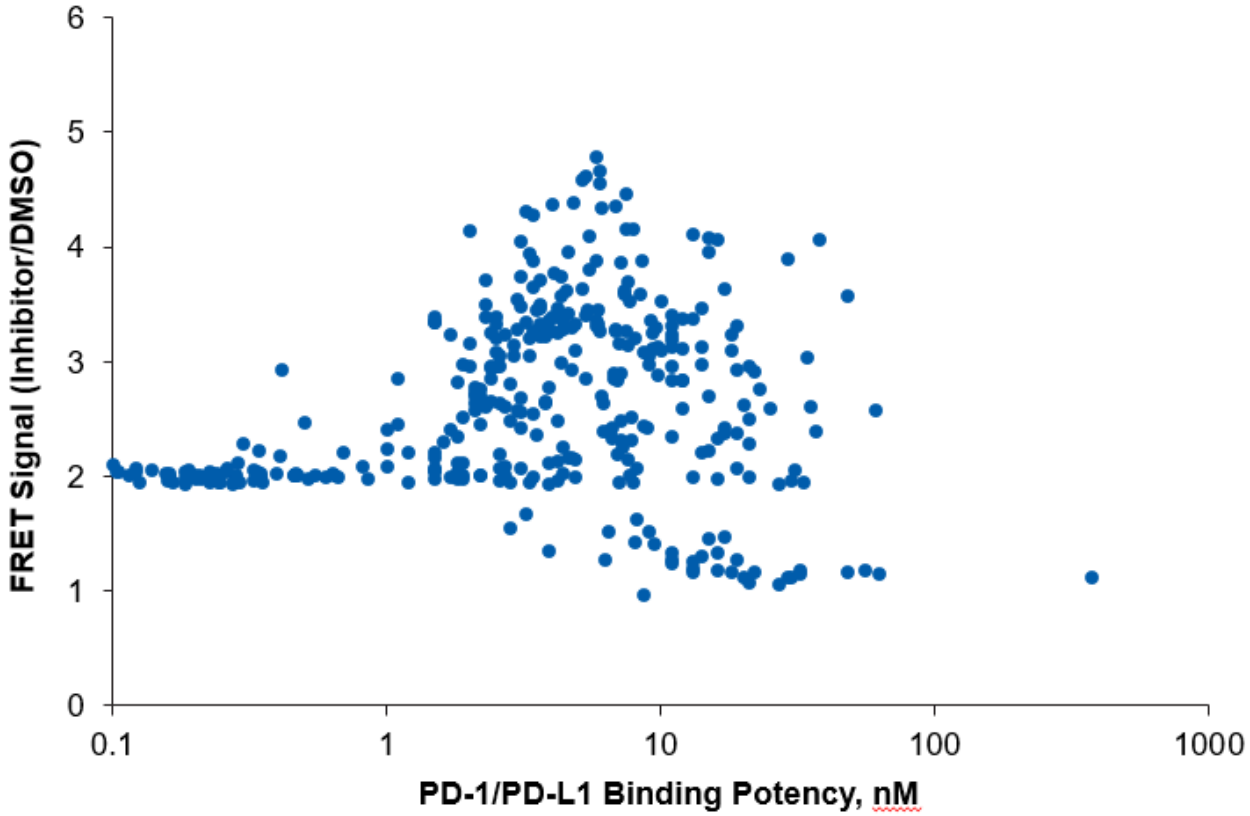


- SEC revealed compound-induced PD-L1 dimers
  - Compounds exhibited variable dissociation during SEC suggesting differences in complex stability
- Thermal shift assays confirmed differences in stability among inhibitor–PD-L1 complexes suggesting alternative binding modes

Compounds Induce Different Conformations of the PD-L1/inhibitor Complex

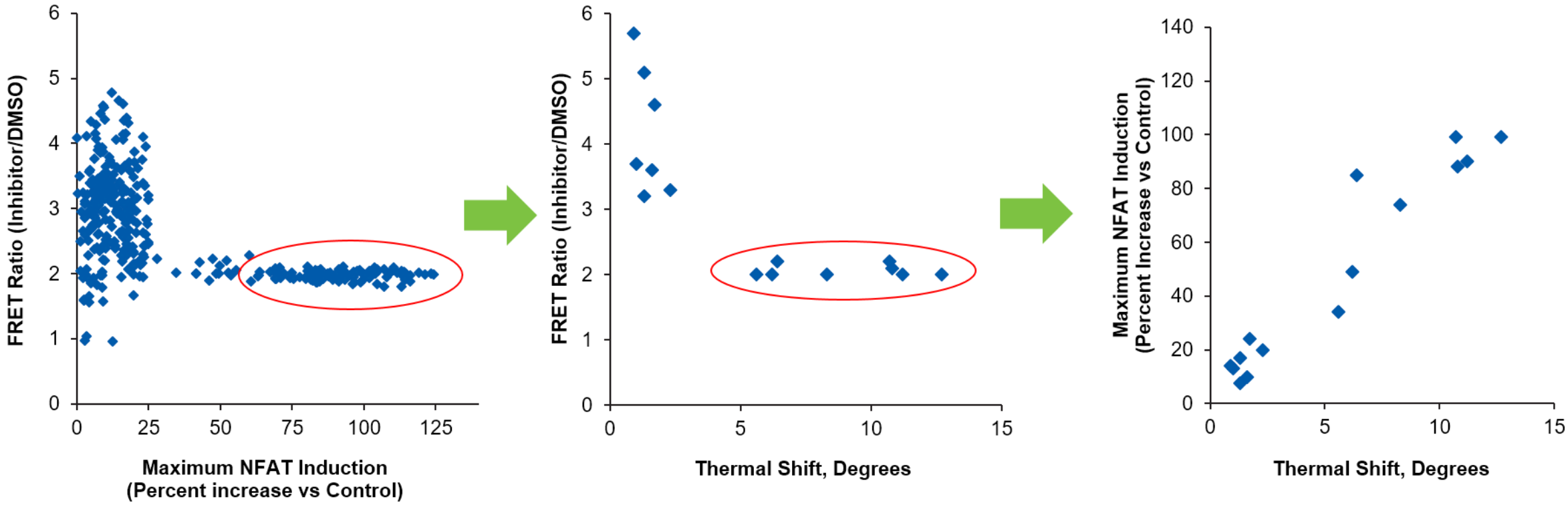


Hinterneder J. Measuring PD-L1 and PD-1 Expression in Human Cells with LANCE Ultra TR-FRET



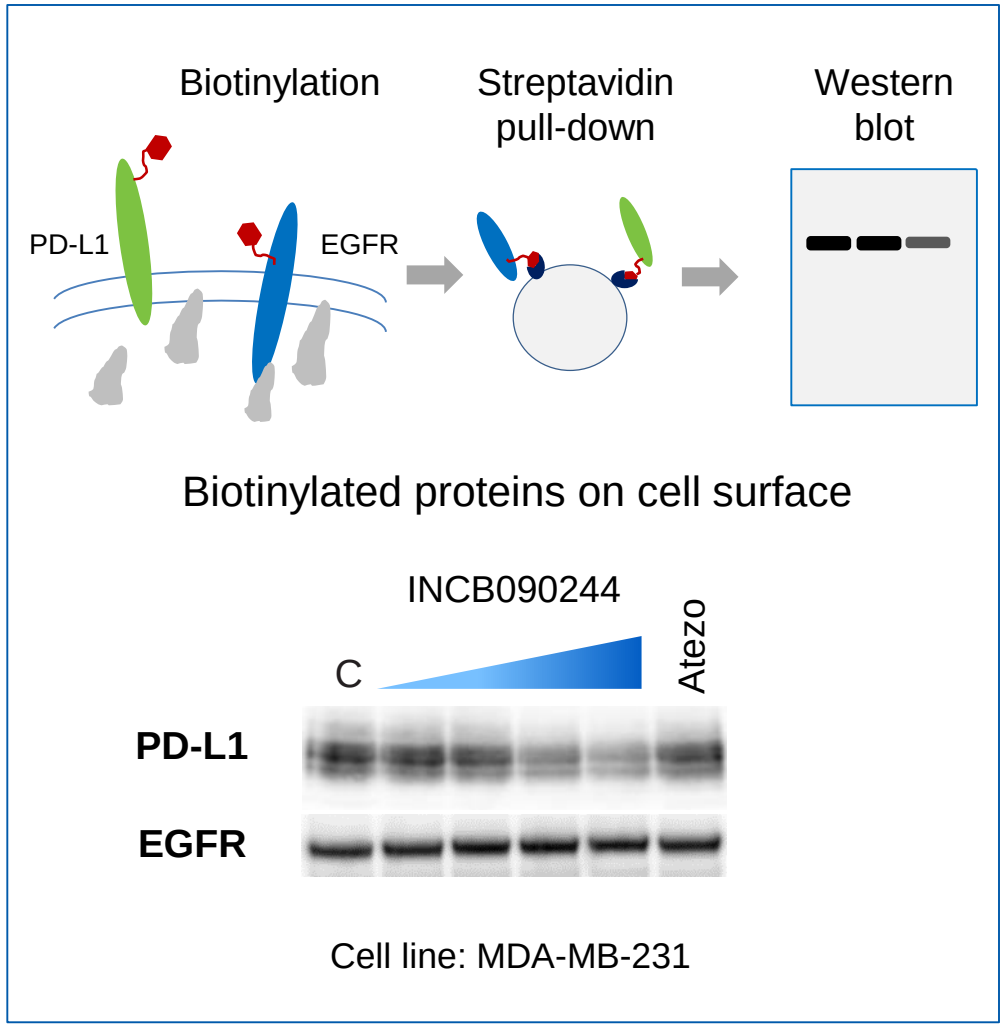
- PD-L1/PD-1 binding potency did not show a correlation with compound-induced dimerization signal

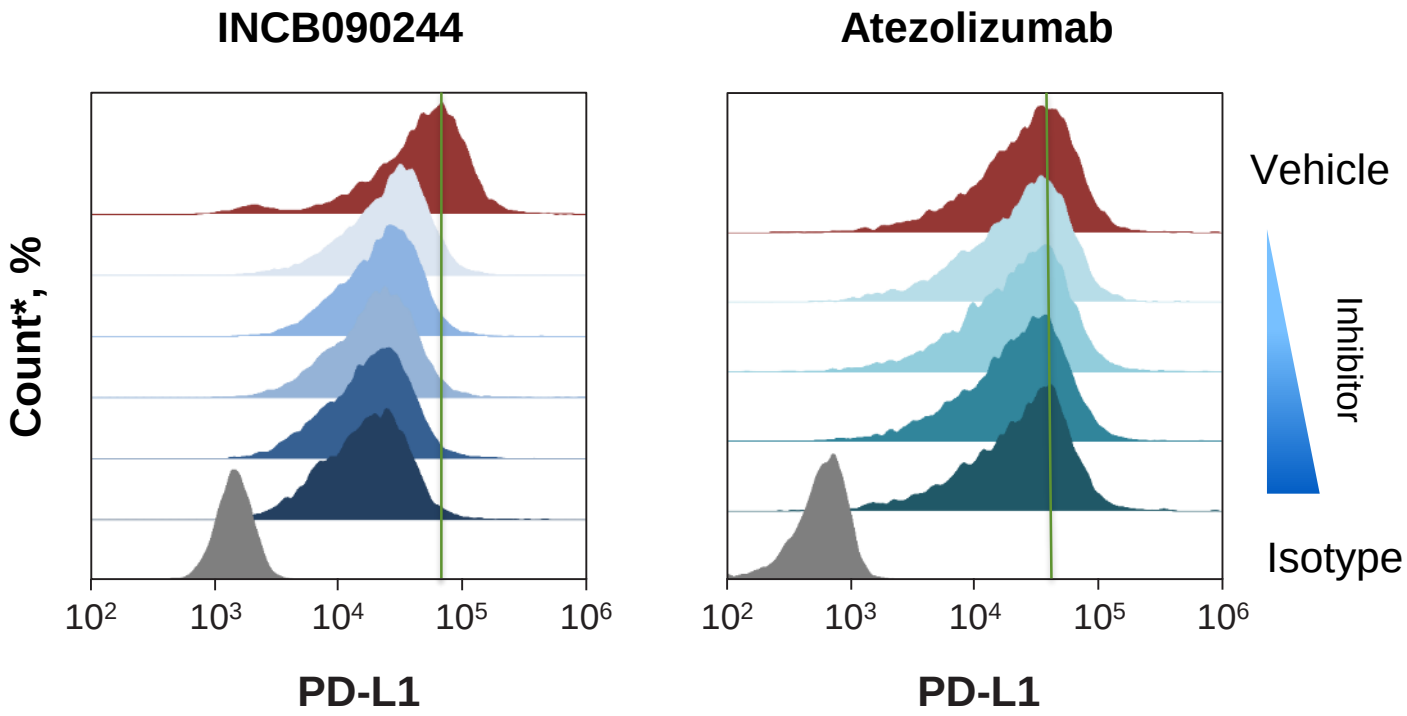
Correlation of Dimerization Signal With Complex Stability and Cellular Activity



- Compounds with high cellular NFAT activity showed a very narrow range of FRET dimerization signal
- Compounds with a FRET ratio of 2 also showed high thermal stabilization and low dimer dissociation during SEC analysis (not shown)
- Cellular activity was correlated with thermal shift data

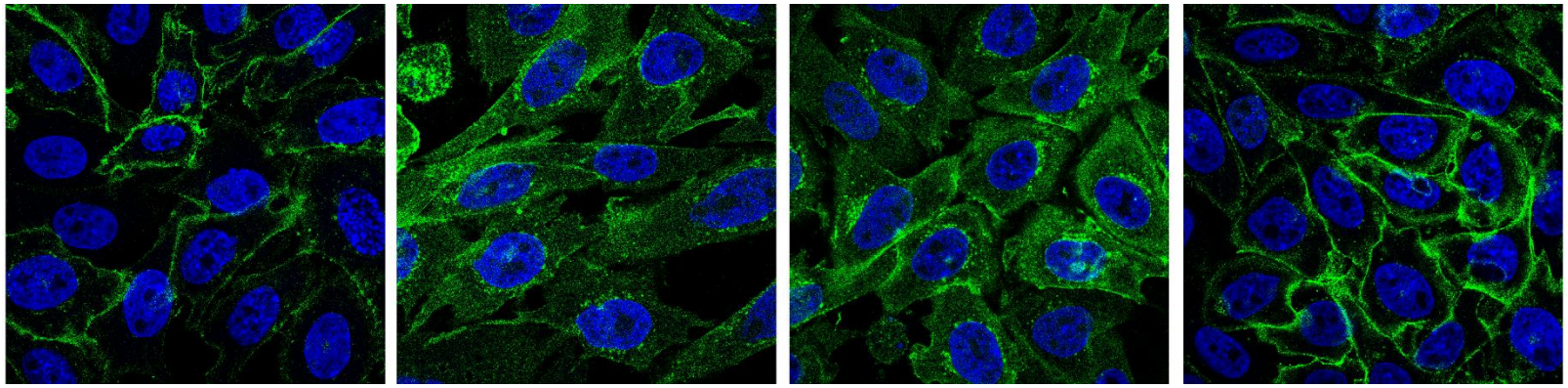
Reduction of Cell Surface PD-L1 by Induced Dimerization





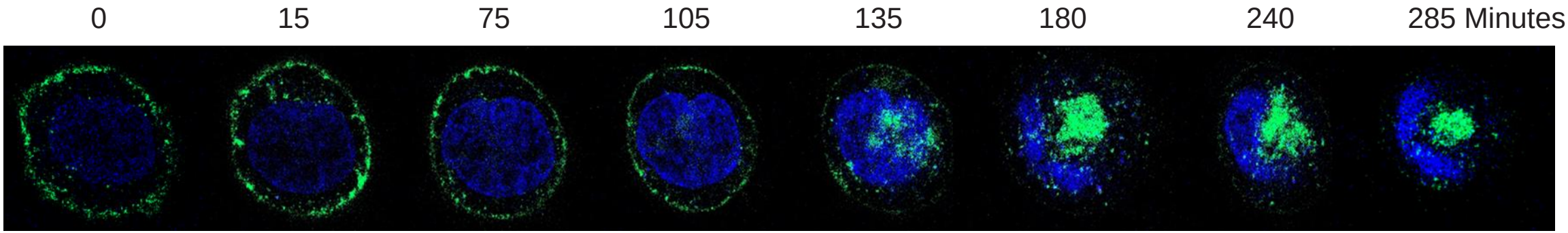
*Normalized to 0–100%

Inhibitor-induced Internalization of PD-L1



- Internalization starts within 1 hour and increases over time
- Co-localization observed with markers of the early endosome

Live cell imaging



Anti-PD-L1–AF488 (Green)
Nuclei – Hoechst (Blue)

Summary

- Rationally designed, competitive small-molecule inhibitors were identified that block PD-1/PD-L1 interactions in biochemical and cellular assays
- Inhibitors that showed functional activity in cells induced dimerization of PD-L1
- Compounds that induced stable dimerization of PD-L1 reduced PD-L1 from the cell surface through a mechanism involving PD-L1 internalization

Acknowledgments

- The authors would like to thank the following individuals for their contributions to the study/presentation: Chao Qi, Neil Lajkiewicz, Zhiyong Yu, Zhenwu Li, Yingda Ye, Leah Konkol, Wenyu Zhu, Liang Lu, Bo Shen, Kai Liu, Colin Zhang, Chunhong He, Dingquan Qian, Meizhong Xu, Song Mei, Jason Wang, Ravi Jalluri, Onur Atasoylu

- Medical writing assistance was provided by Sneha DSilva, MD, of Envision Pharma Group (Philadelphia, PA), funded by Incyte Corporation