AACR-NCI-EORTC Virtual International Conference on
MOLECULAR TARGETS AND CANCER THERAPEUTICS
October 7-10, 2021

Poster #P249

Preclinical Antitumor Activity of NVL-520 in Patient-Derived Models Harboring ROS1 Fusions, Including G2032R Solvent Front Mutation

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Henry E. Pelish

I am an employee of Nuvalent.
I will not discuss off-label use and/or investigational use in my presentation.

NVL-520: a Potential Next-Generation ROS1 Inhibitor

NVL-520 was designed to address clinical challenges of existing agents used to treat ROS1+ disease, including:

1. **Activity against ROS1 kinase**
   ROS1-fusion is an oncogenic driver found in up to 3% of non-small cell lung cancer (NSCLC)
2. **Activity against ROS1 resistance mutations, especially G2032R solvent front mutation**
   G2032R solvent-front mutation arises in response to various ROS1 TKIs
3. **Activity in the central nervous system (CNS)**
   up to 40% of patients with ROS1+ NSCLC present with brain metastases
4. **Sparing TRKB**
   a key off-target kinase of some ROS1 inhibitors believed to contribute to treatment-related adverse events

Pelish *et al.* AACR Annual Meeting 2021

NVL-520 Inhibited ROS1-Dependent Cell Viability and Signaling *In Vitro*

Potency color legend
IC_{50} < 10 nM
10 nM ≤ IC_{50} < 100 nM
IC_{50} ≥ 100 nM

CELL VIABILITY INHIBITION

Model name	Alteration	Crizotinib	Entrectinib	NVL-520	Repotrectinib	Taletrectinib	Lorlatinib	Cabozantinib
MGH193-1 PDC	EZR-ROS1	19	9.2	1.0	2.3	5.3	0.41	18
Ba/F3	CD74-ROS1	40	23	1.2	4.3	20*	1.1	-
MGH9018-1 PDC	CD74-ROS1 G2032R	1000	590	4.7	30	250	460	49
Ba/F3	CD74-ROS1 G2032R	960	1500*	3.5	25	100	310	-

Table 1 | Cell viability 5-day IC_{50} (nM) of patient-derived cell lines (PDC) or 3-day IC_{50} (nM) of Ba/F3 cell lines expressing ROS1 fusions. All values have been repeated (n=2 testing or more) except for values indicated with *, which have been tested once (n=1 testing).

SIGNALING PATHWAY INHIBITION

MGH193-1 PDC
EZR-ROS1

MGH9018-1 PDC
CD74-ROS1 G2032R

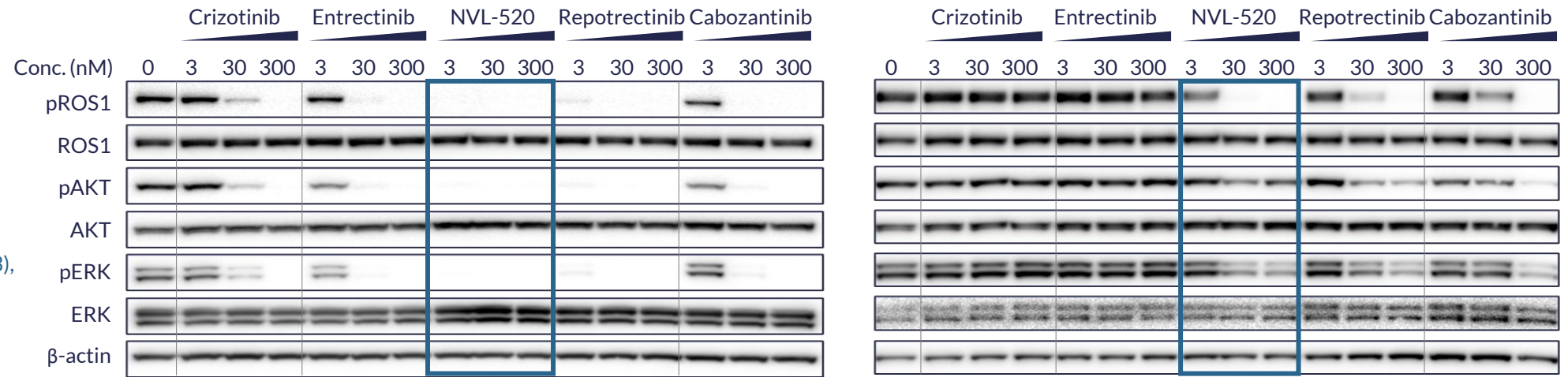
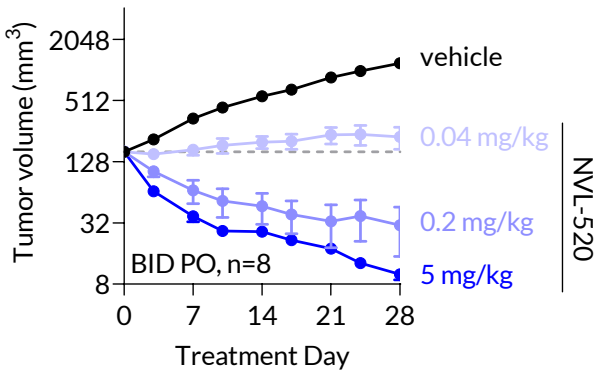


Figure 1 | Signaling pathway analysis by western blot in patient-derived cell lines (PDC) expressing ROS1 fusions treated for 6 hours. Phosphorylation sites are pROS1 (Y2274), pAKT (S473), and pERK (T202/Y204).

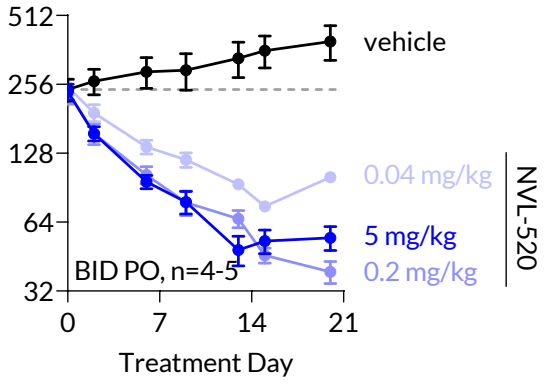
NVL-520 Induced Regression In Patient-Derived Xenograft Models

ROS1, WILD-TYPE KINASE DOMAIN

1 *Lu01-0414 PDX*
SDC4-ROS1

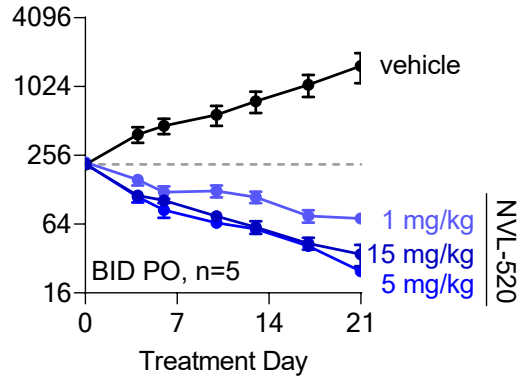


2 *CTG-0848 PDX*
CD74-ROS1

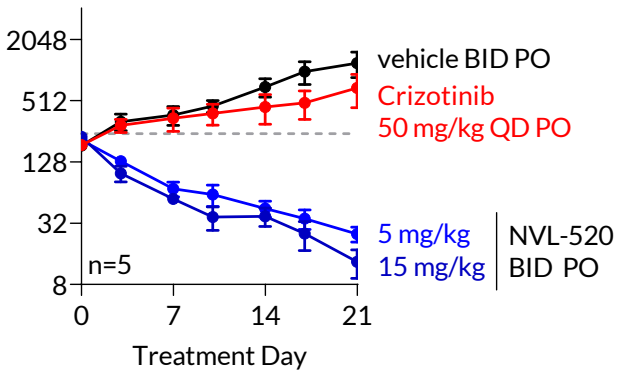


ROS1, MUTANT KINASE DOMAIN

3 *CTG-2532 PDX*
CD74-ROS1 G2032R



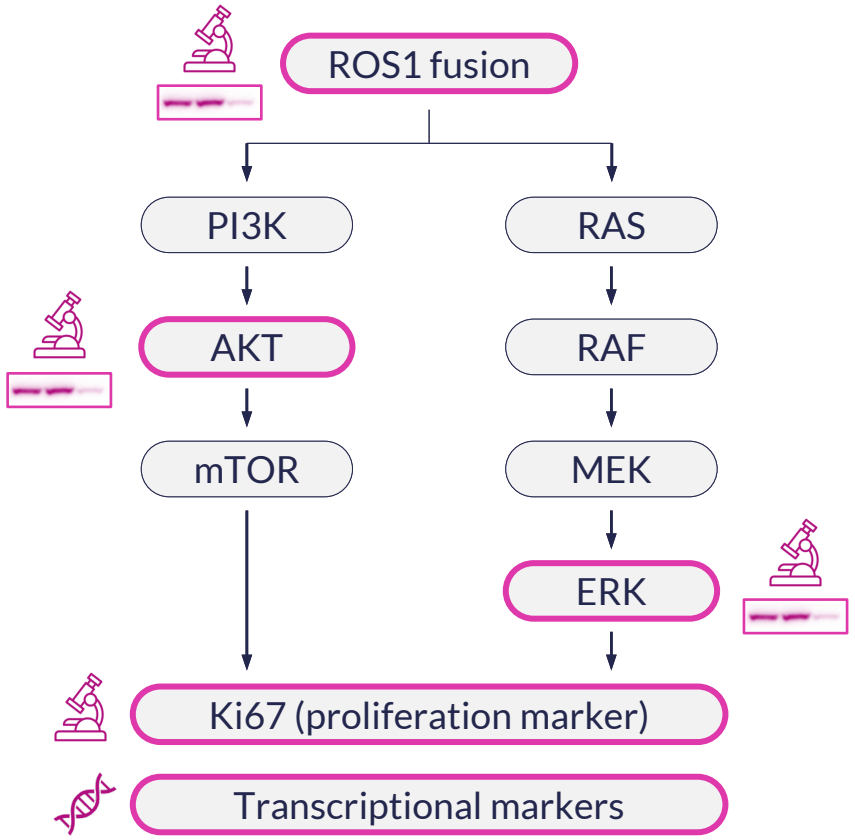
4 *MGH9018-1 PDX*
CD74-ROS1 G2032R



Three models were used for pharmacodynamics studies (BID × 5 days)

Figure 2 | Activity of NVL-520 in various ROS1 patient-derived xenograft (PDX) models subcutaneously implanted in Balb/c mice or NSG mice. Vehicle is 20% HP-β-CD. Average ± SEM plotted. No significant changes in body weight were observed (data not shown). NVL-520 induced regression irrespective of fusion partner (SDC4 or CD74).

Overview of Tumor Pharmacodynamics Studies



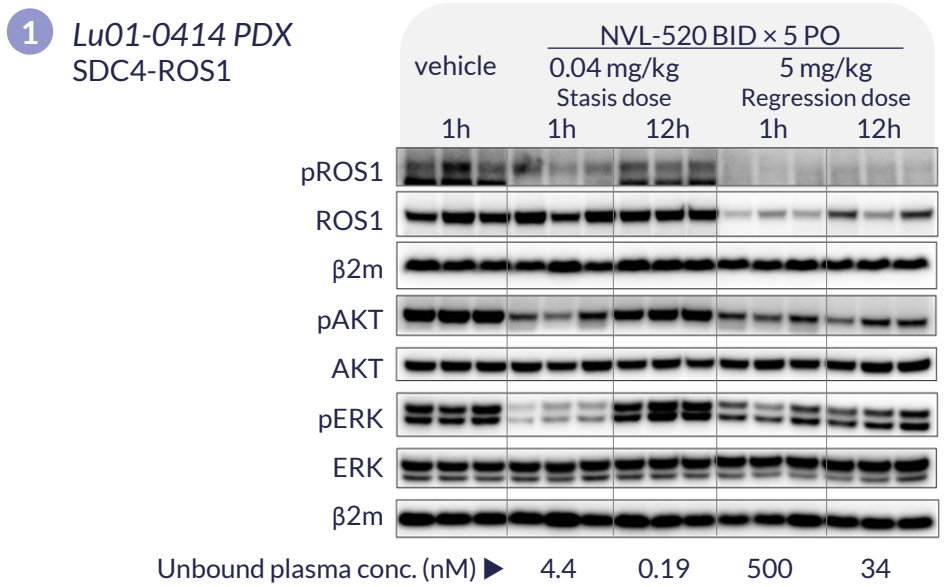
Western blot

Immunohistochemistry (IHC)

Gene expression profiling

Figure 3 | ROS1 fusions signal through the PI3K and the MAP kinase pathways to effect proliferation and other transcriptional changes. Some members of these pathways may serve as pharmacodynamic biomarkers of response to NVL-520. In subsequent slides, we present the response of potential biomarkers shown in pink to NVL-520 treatment. Responses were measured using western blot, IHC, and/or gene expression profiling.

NVL-520 Inhibited ROS1 Signaling in Tumors, Western Blot Analysis



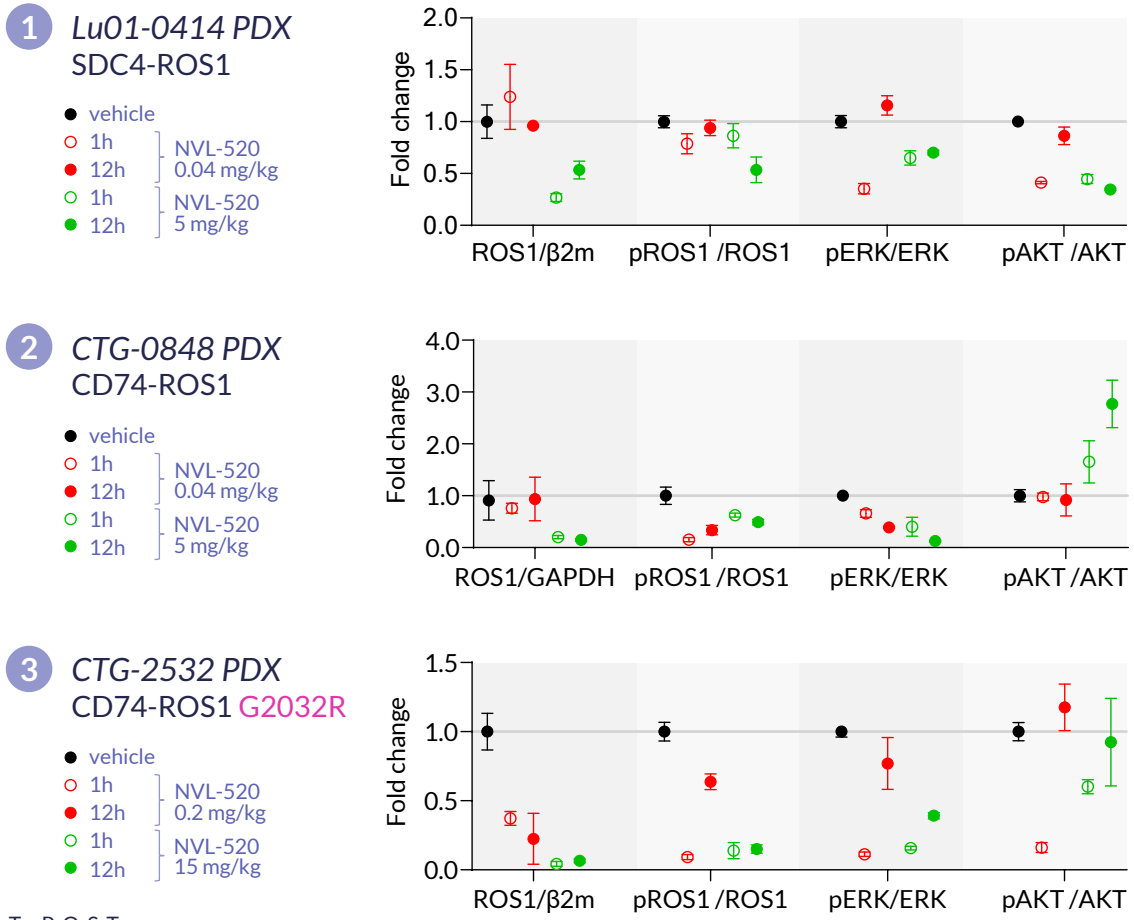


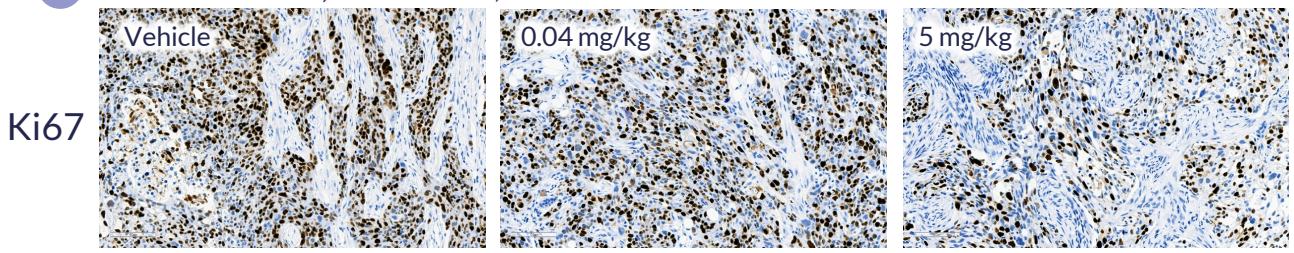
Figure 4 | (**Left**) Representative western blot of Lu01-0414 PDX treated with NVL-520 (BID PO) revealed suppression of ROS1-dependent cell signaling through reduced levels of pROS1, ROS1, pAKT, and pERK. Pharmacokinetic analysis indicated dose-dependent differences in NVL-520 unbound plasma concentrations. In this model, 0.04 mg/kg achieved stasis and 5 mg/kg achieved regression (see Figure 2). Reduction in total ROS1 has been previously reported for crizotinib and taletrectinib in a cell line model[1]. (**Right**) Western blot quantitation of three PDX models treated with NVL-520 (BID PO) confirmed reduced levels of ROS1, pROS1, and pERK. Y-axis denotes fold-change normalized to vehicle treatment, indicated with black dots and horizontal grey line. Mean ± SEM plotted.

[1]Katayama *et al. Nature Communications* 2019, 10:3604.

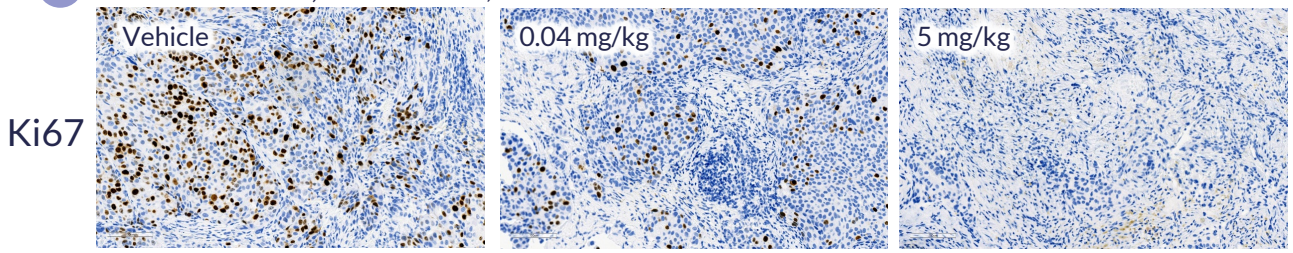
NVL-520 Inhibited ROS1 Signaling in Tumors, IHC analysis

Figure 5 | (**Left**) Representative Ki67 IHC staining of tumor sections. Scale bar = 100 micron. (**Right**) Quantitative digital image analysis. Mean ± SEM plotted. Effects on pERK, ERK and pAKT were model-dependent.

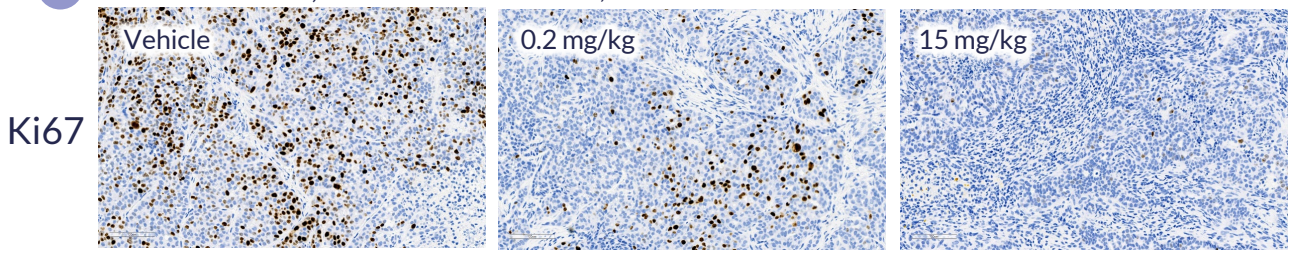
1 *Lu01-0414 PDX*, SDC4-ROS1, NVL-520 BID × 5 PO

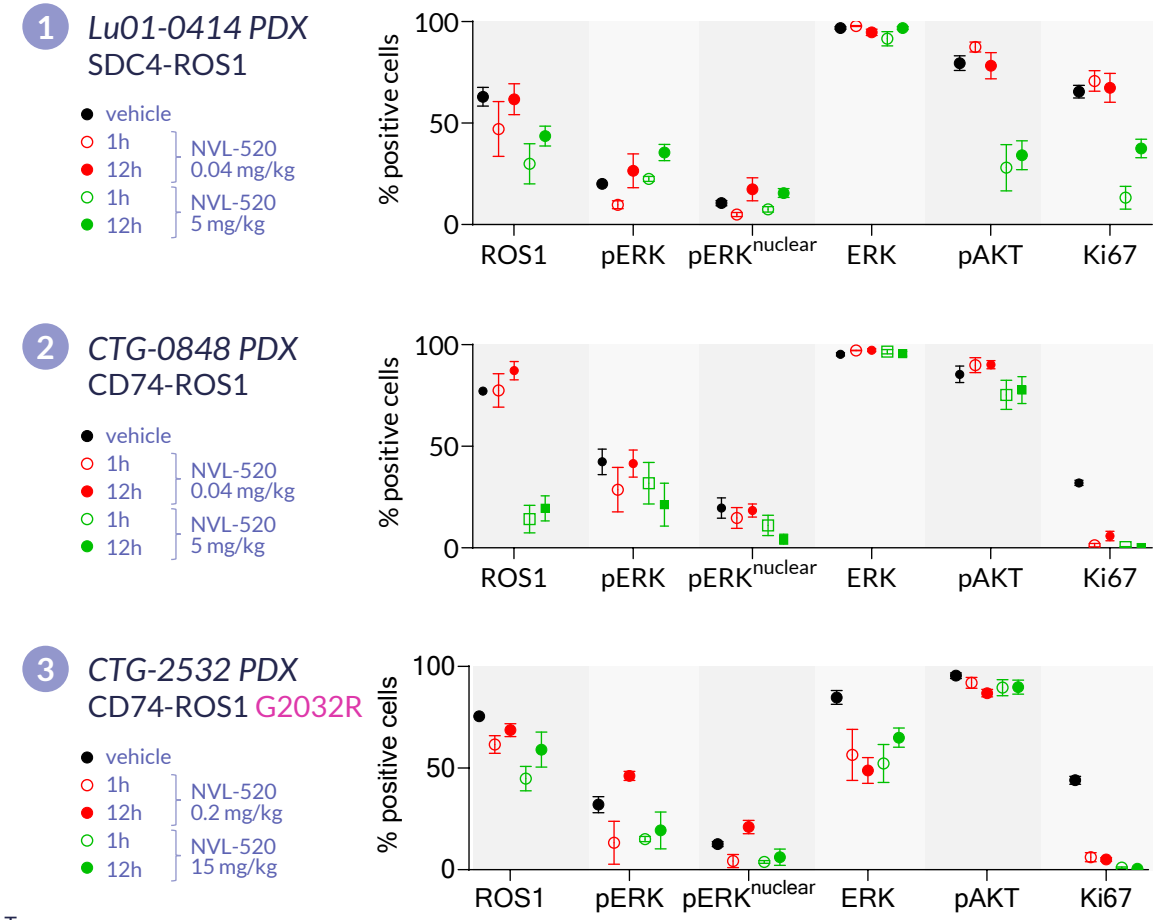


2 *CTG-0848 PDX*, CD74-ROS1, NVL-520 BID × 5 PO



3 *CTG-2532 PDX*, CD74-ROS1 G2032R,NVL-520 BID × 5 PO





NVL-520 Reduced Expression Levels of MAP Kinase Pathway Genes in Tumors

NVL-520 BID × 5, PO

1 *Lu01-0414 PDX*
SDC4-ROS1

2 *CTG-0848 PDX*
CD74-ROS1

3 *CTG-2532 PDX*
CD74-ROS1 G2032R

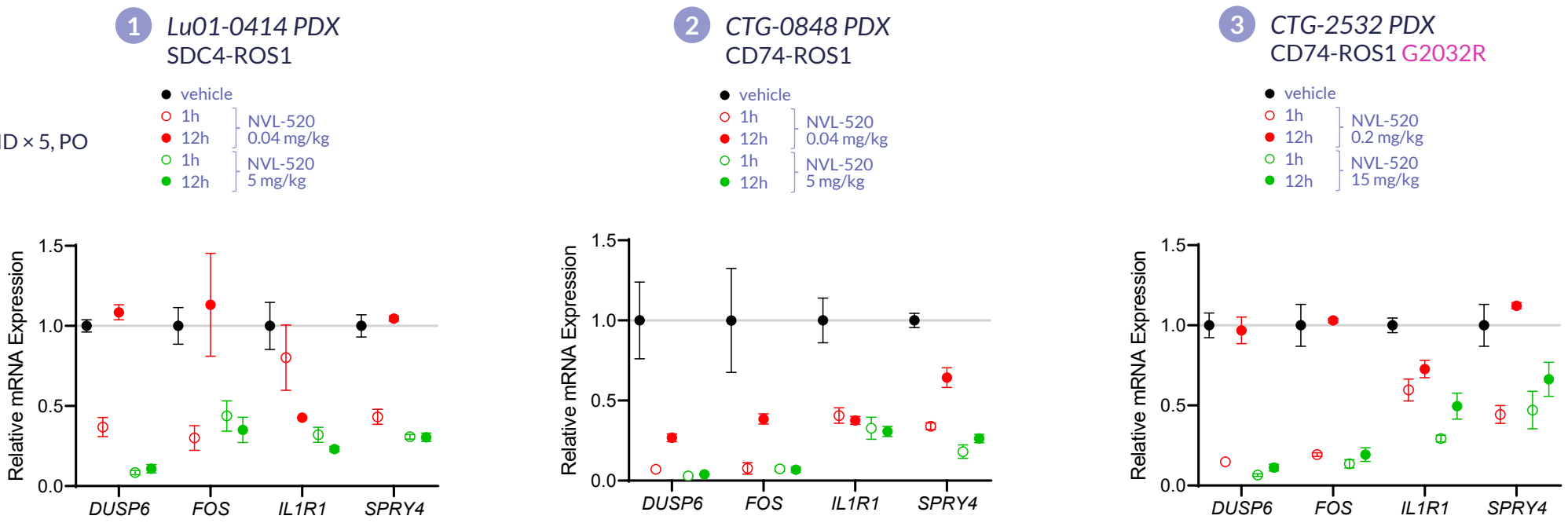


Figure 6 | Gene expression profiling of tumor samples with the NanoString 770 gene nCounter PanCancer Pathways panel. Expression levels of MAP kinase pathway genes *DUSP6*, *FOS*, *IL1R1*, and *SPRY4* were dose-dependently reduced in the tumors upon NVL-520 treatment. The Y-axis denotes relative expression normalized to vehicle treatment, indicated with black dots and horizontal grey line. Mean ± SEM plotted.

Conclusions

- NVL-520 demonstrated potent activity against multiple ROS1+ NSCLC patient-derived *in vitro* cell line (PDC) and *in vivo* xenograft (PDX) models. Activity was observed:
  - irrespective of fusion partner (SDC4 or CD74)
  - against both wild-type and TKI-resistant solvent front mutant (G2032R) ROS1-kinase domain
- Pharmacodynamic analysis of tumors from mice treated with NVL-520 revealed a dose-dependent reduction in ROS1 levels, markers of downstream signaling, and cell proliferation across multiple models of ROS1-driven disease.