

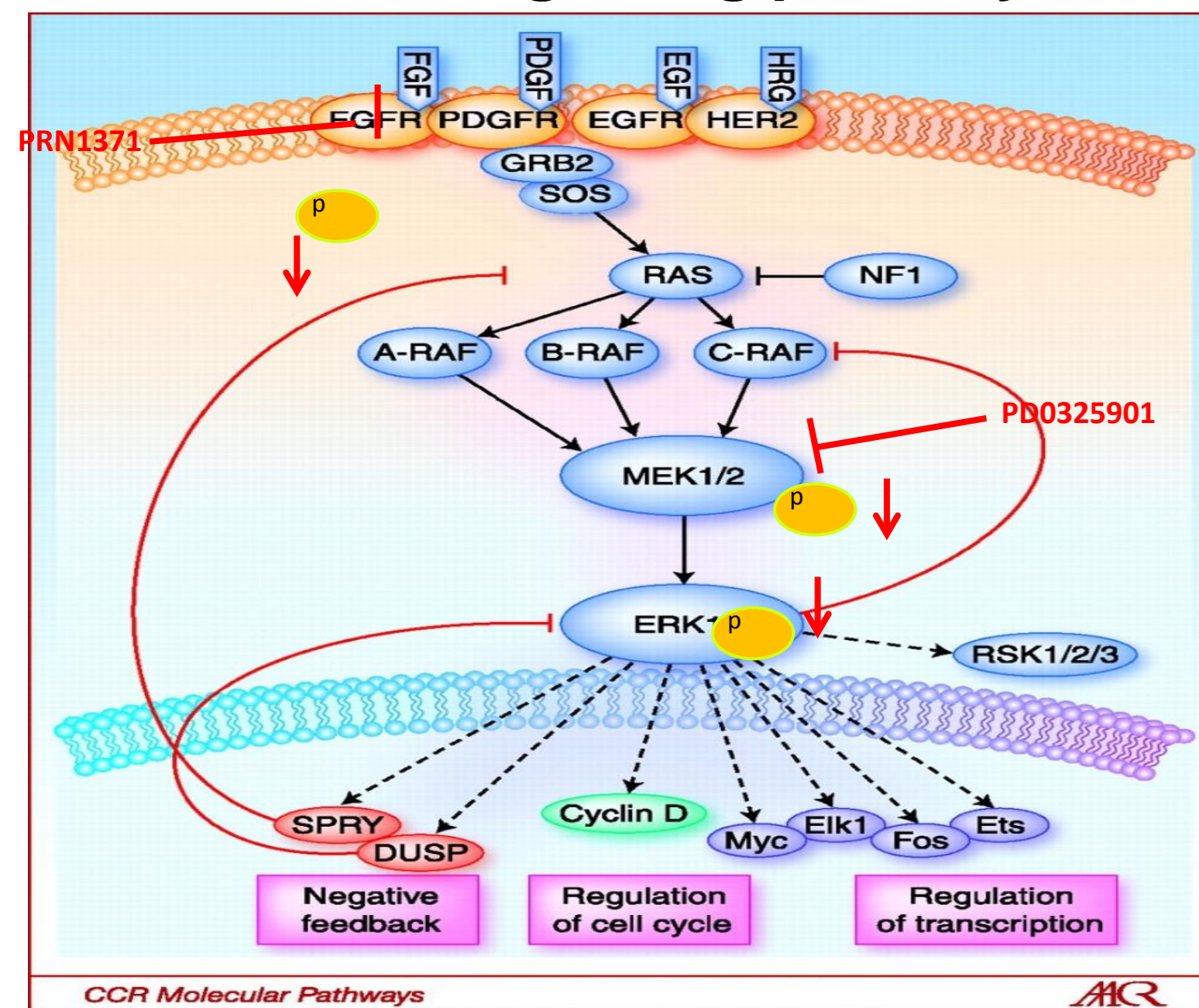
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Introduction

Multiple human cancers harbor alterations in FGFRs that drive tumor growth, including mutations, translocations and amplifications. PRN1371 is an irreversible, covalent, FGFR1-4 inhibitor that exhibits highly selective and sustained inhibition of FGFR which extends well beyond circulating drug concentrations. As PRN1371 exhibits a covalent mechanism of action, the duration of inhibition of the FGFR signaling pathway is dependent on protein turnover of FGFR. Different FGFR alterations may exhibit different rates of protein turnover. This may impact dosing regimen of PRN1371 in the clinic. Thus, we set out to investigate duration of pathway inhibition across cancer cell lines of various lineages harboring different FGFR alterations.

FGFR signaling pathway



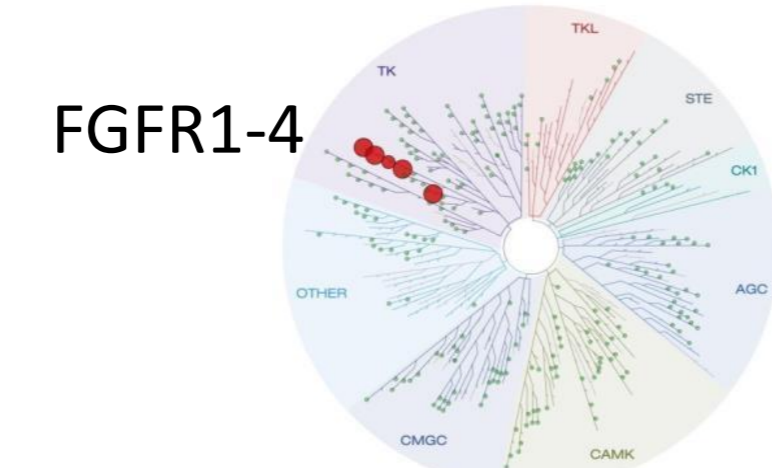
Pratillas et al., 2010

Materials and Methods

- Three cancer cell lines harboring different FGFR alterations were assessed for pERK EC₅₀ of PRN1371 and PD0325901 (Pfizer) using alpha-screen assay.
- Cancer cell lines were treated with increasing concentrations of PRN1371 *in vitro* for 1 hour, before compound was washed out.
- Doses for treatment period of the washout experiments were selected based on pERK EC₅₀ and included 10x, 100x and 1,000x EC₅₀ pERK (saturating dose).
- Cells were maintained media containing 10% FBS. For washout experiments, RT4 cells were incubated overnight in low-serum conditions (media containing 1% FBS). Cells were stimulated with bFGF at 50 ng/ml in final 20 min of either 1 hr treatment or washout period before protein lysates were harvested and pERK probed using western blot analysis.

PRN1371 is highly potent and selective FGFR inhibitor

| Assay | PRN1371 |
|--|---------|
| Biochemical IC50 (nM) | |
| FGFR1 | < 1 |
| FGFR2 | 1 |
| FGFR3 | 4 |
| FGFR4 | 20 |
| VEGFR | >500 |
| 250 kinase panel (# of kinases) | |
| 90% inh @ 1 μM | 5 |
| 50% inh @ 1 μM | 14 |



Kinase selectivity profile of PRN1371 screened against 250 kinases. Dots represent individual kinase with > 90% inhibition at 1 μM.

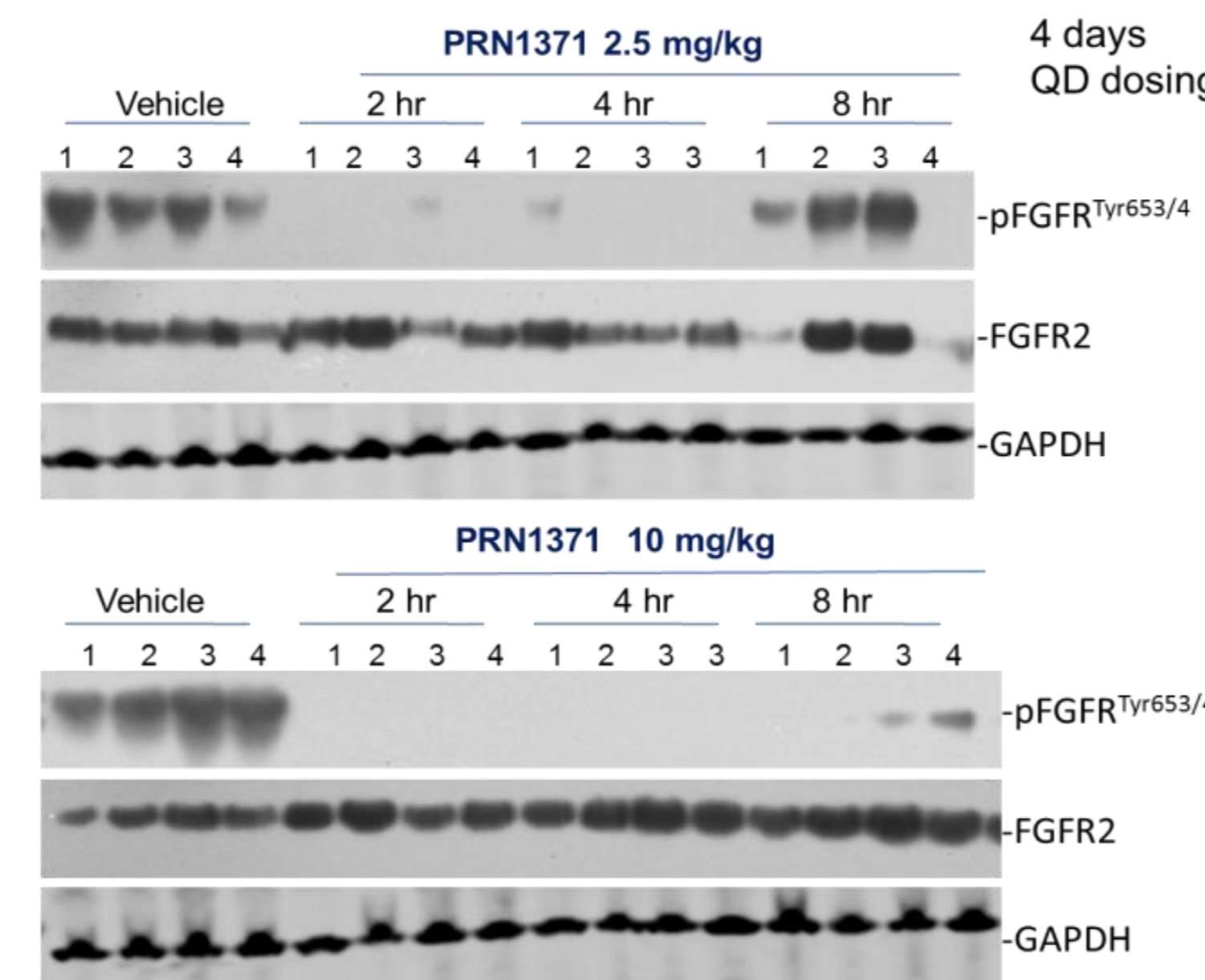
PRN1371 maintains high potency against FGFR alterations

| Assay | PRN1371 | BGJ398 | AZD4547 |
|-------------------------------------|---------|--------|---------|
| Transfected Ba/F3 IC50 (nM) | | | |
| FGFR2 WT | < 1 | 7.5 | 11 |
| FGFR2 (K660E) | 1 | 14 | 9.8 |
| FGFR2 (K660N) | < 1 | 6.9 | 11 |
| FGFR2 (N550K) | 4 | 87 | 121 |
| FGFR3 WT | 2 | 7.4 | 37 |
| FGFR3 (K650M) | 3 | 45 | 133 |
| Cell proliferation IC50 (nM) | | | |
| SNU16 (FGFR2 amp) | 3 | 5 | 7 |
| RT4 (FGFR3:TACC3) | 4 | 184 | 230 |
| RT112 (FGFR3:TACC3) | 4 | 19 | 36 |
| AN3CA (FGFR2 mut) | 43.3 | n/a | n/a |
| Hep3B (FGFR4; FGF19) | 6 | 96 | 116 |
| OPM2 (FGFR3 transl) | 14 | n/a | n/a |

Cellular activity was assessed in range of BaF/3 cells transfected with FGFR or cancer cell lines exhibiting FGFR alterations.

Results

Sustained pFGFR inhibition in SNU16-tumor bearing mice despite rapid clearance

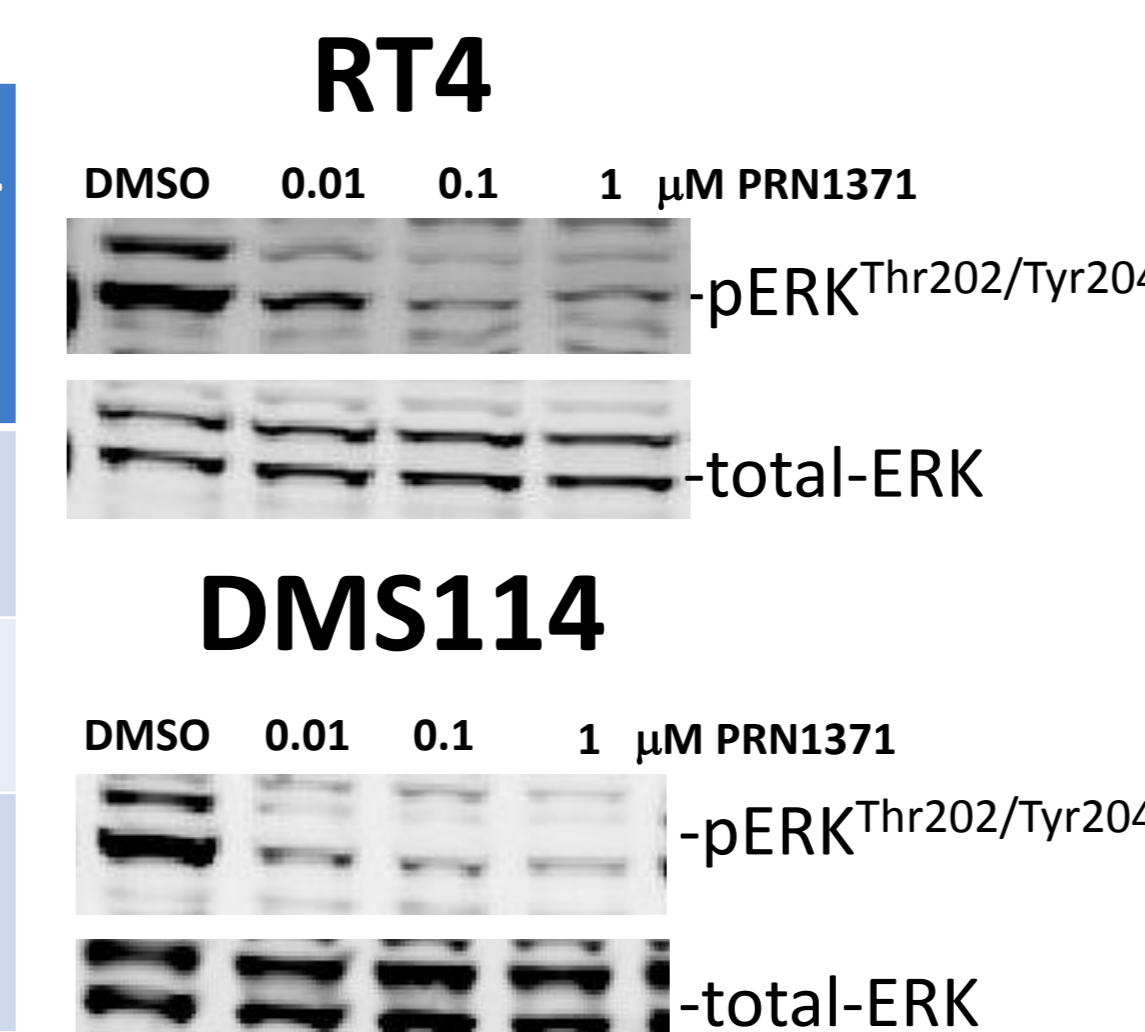


| Time pt (hr) | Average plasma conc (ng/ml) Dose: 2.5 mg/kg | Average plasma conc (ng/ml) Dose: 10 mg/kg |
|--------------|---|--|
| 2 | 8.1 | 68 |
| 4 | BLQ | 2.5 |
| 8 | BLQ | BLQ |
| 12 | BLQ | BLQ |

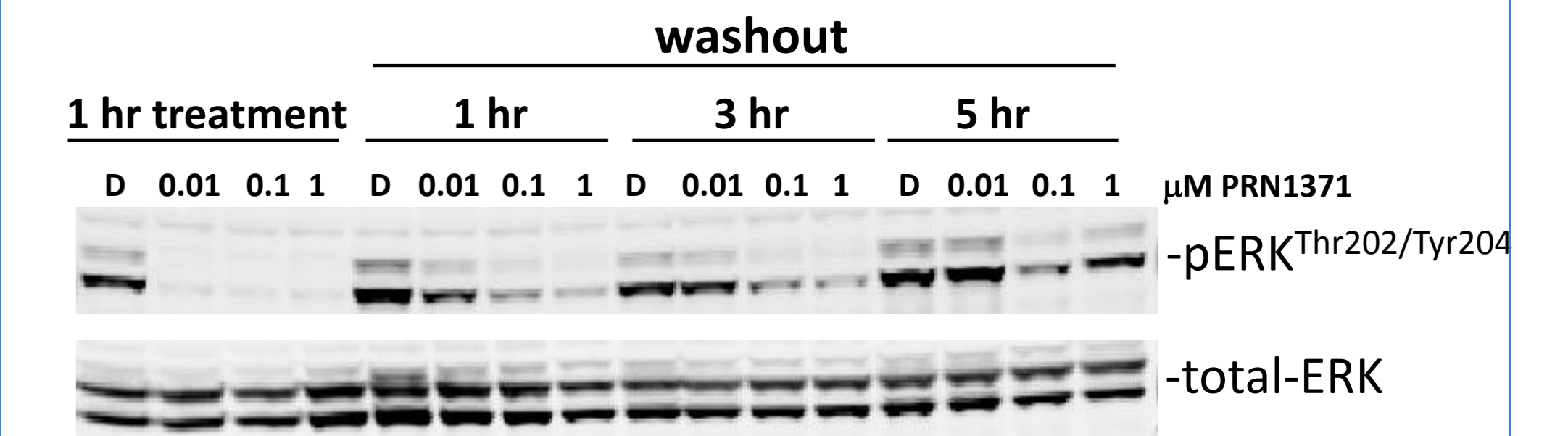
BLQ: Below the Limit of Quantitation

Comparable pERK EC₅₀ observed across different cancer cell lines

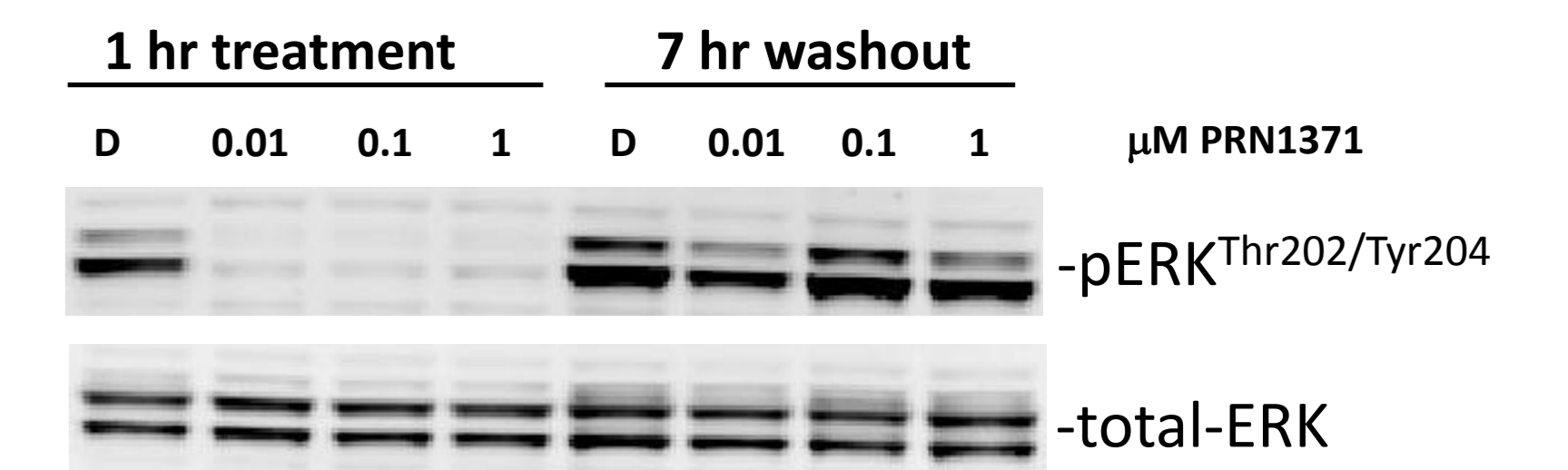
| FGFR alteration | Lineage | Cell line | PRN1371 pERK EC ₅₀ (nM) | PD0325901 pERK EC ₅₀ (nM) |
|---------------------------|---------------|-----------|------------------------------------|--------------------------------------|
| FGFR3-TACC3 | Urinary tract | RT4 | 0.64 | 0.41 |
| FGFR1 CN 10 | Lung | DMS114 | 0.91 | 0.70 |
| FGFR2 K310R, N549K, N550K | Endometrial | AN3CA | 0.76 | 0.70 |



pERK rebounds 5 hrs post washout in RT4 cells



Full rebound of pERK 7 hrs post washout in RT4 cells



D: DMSO

In vivo studies confirm that BID > QD for inhibiting tumor growth in xenograft models

| Tumor | Doses of PRN1371 | Tumor growth inhibition (TGI%) |
|-------|------------------|--------------------------------|
| SNU16 | 10 mg/kg QD | 39% |
| | 5 mg/kg BID | 50% |
| RT4 | 10 mg/kg BID | 68% |
| | 10 mg/kg QD | 29% |
| | 2.5 mg/kg BID | 39% |
| | 5 mg/kg BID | 47% |

Conclusions

- PRN1371 is a potent, highly selective irreversible FGFR1-4 inhibitor exhibiting sustained inhibition of FGFR signaling.
- Sustained pFGFR inhibition observed in SNU16-tumor bearing mice despite rapid clearance of PRN1371.
- Time and concentration-dependent rebound of pERK detected in RT4 cells harboring FGFR3:TACC3 fusion.
- Preclinical washout data and *in vivo* xenograft data suggests that different dosing regimens of PRN1371 could be considered based on the protein turnover of different FGFR alterations.
- Phase I clinical trial of PRN1371 for the treatment of solid tumors is ongoing (NCT02608125).