Discovery and Characterization of the Potent, Allosteric SHP2 inhibitor GDC-1971 for the Treatment of RTK/RAS Driven Tumors

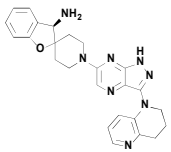
Bret Williams[1], Alexander Taylor[1], Olivia Orozco[1], Christopher Owen[1], Elizabeth Kelley[1], Andre Lescarbeau[1], Kelley Shortsleeves[1], Randy Kipp[1], Vy Nguyen[1], Erin Brophy[1], Jeremy Wilbur[1], Yong Tang[1], David Lanzetta[1], Nigel Waters[1], Sherri Smith[1], Fabrizio Giordanetto[2], Paul Maragakis[2], Jack Greismann[2], Lindsay Willmore[2], Eric Therrien[3], Yang Xiao[5], Marie Evangelista[5], Luca Gerosa[5], Eva Lin[5], Mark Merchant[5], Alfonso Arrazate[5], Emily Chan[5], Pablo Sáenz-López Larrocha[5], Stefan Chun[5], Thomas Hunsaker[5], Gauri Deshmukh[5], Christine M. Bowman[5], David E Shaw [2,4], Mark Murcko[1], Mahesh Padval[1], W Patrick Walters[1], James Watters[1], Donald A Bergstrom[1]

[1]Relay Therapeutics, Cambridge, MA; [2]D. E. Shaw Research, New York, NY; [3]Schrödinger Inc., New York, NY; [4]Department of Biochemistry and Molecular Biophysics, Columbia University, New York, NY [5]Genentech, South San Francisco, CA

INTRODUCTION

The non-receptor protein tyrosine phosphatase SHP2 (PTPN11) plays an important role in the regulation of RAS/MAPK signal transduction downstream of growth factor receptor activation. GDC-1971 (formerly RLY-1971), is a highly potent, selective, and orally bioavailable small-molecule SHP2 inhibitor that stabilizes SHP2 in a closed, auto-inhibited conformation. GDC-1971 inhibits both wild-type SHP2 (IC50 0.7nM) and the E76K activating mutant (IC50 230nM) in biochemical assays. In standard 2-dimensional and anchorage-independent growth conditions, GDC-1971 inhibits cellular proliferation in models harboring receptor tyrosine kinases (RTKs), SHP2, or KRAS mutations in a dose-dependent manner. GDC-1971 potently inhibits the proliferation of cellular models harboring KRAS G12C or G12A mutations (median IC50 <80 nM) compared to models harboring other KRAS G12, G13 or Q61 mutations (median IC50 >1 uM), indicating a link between KRAS GTP hydrolysis and SHP2 dependency. In vivo, GDC-1971 demonstrates dose-dependent RAS/MAPK pathway inhibition and induces significant tumor-growth inhibition in human xenograft models harboring EGFR and KRAS alterations at continuous daily doses that are well tolerated. GDC-1971 also displays significant synergy in combination with other targeted therapies in cell line models. GDC-1971 in combination with the KRAS G12C inhibitor GDC-6036 resulted in significant tumor regression in a KRAS G12C-mutant NSCLC xenograft model at doses where single agent treatment showed only modest tumor growth inhibition. In rodent and dog toxicology studies, GDC-1971 is well tolerated at exposures above those required to induce regression in xenograft models. Continuous daily dosing of GDC-1971 is being studied in combination with GDC-6036 in the clinic (NCT04449874).

GDC-1971



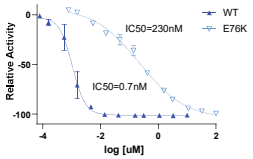
Pharmacokinetic Properties of GDC-1971

PK	Mouse	Rat	Dog
CL (CL_p) (ml/mn/kg)	18 (782)	1.9 (475)	7.9 (303)
VD_{SS} (L/kg	1.8	0.39	4.8
$t_{1/2}$ (h)	3.2	3.2	11
F (%) (@ 10 npk)	28	97	60
PPB (% unbound)	2.3	0.4	2.6
@ 10 mpk, t (h with $C_{plasma,\ unbound}$ > cell IC_{50}	3	14	
Blood $K_{WB/PL}$	1.0	0.75	0.8

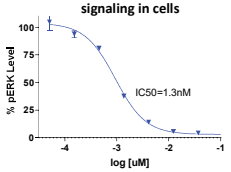
RESULTS

1. GDC-1971 is a potent SHP2 inhibitor

A. In vitro inhibition of WT and E76K SHP2



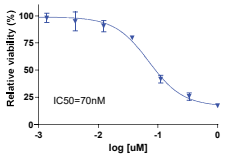
B. GDC-1971 inhibits MAPK signaling in cells



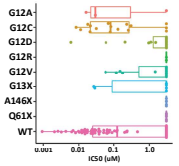
A. Activity of SHP2 was monitored using the surrogate substrate DiFMUP following the incubation of recombinant SHP2 with activating peptide. **B.** SHP2 inhibition was determined by pERK AlphaLISA Assay following 2hr incubation of KYSE520 cells with GDC-1971.

2. GDC-1971 inhibits the proliferation of tumor cell lines in vitro

A. Treatment with GDC-1971 inhibits growth of an EGFR-amplied model



B. GDC-1971 is active in KRAS G12A/C and a subset of KRAS WT cell line models

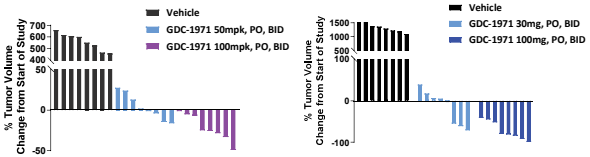


A. Relative viability was determined in KYSE520 cells treated with GDC-1971 in a 2D growth assay. Exponentially dividing cells were exposed to compound for 4 days, cell viability was measured using CellTiter-Glo. **B.** 166 cell lines were grown in ultra low-attachment (ULA) plates and spheres were allowed to form. Spheres were treated with GDC-1971 for 7 days and viability was determined by CellTiter-Glo.

3. GDC-1971 inhibits the growth of RTK and KRAS mutant tumor models in vivo

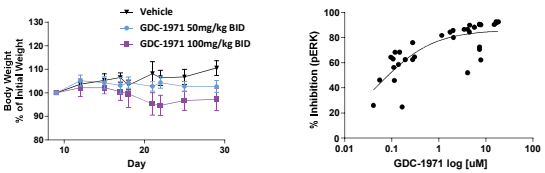
A. KYSE520 EGFR-amplified Esophageal

B. NCI-H358 KRAS G12C NSCLC



C. Body Weight

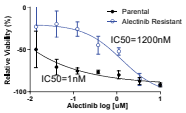
D. In vivo PK/PD Relationship



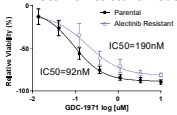
Dosed orally, GDC-1971 demonstrates potent growth inhibition in a dose-dependent manner leading to complete regressions at the top doses tested. Xenografts were established in BALB/c Nude mice and treated for 4 weeks with compound. **A.** KYSE520, an EGFR-amplified esophageal squamous cell carcinoma **B.** NCI-H358, KRAS G12C mutant Non-Small Cell Carcinoma **C.** GDC-1971 is well tolerated with no significant body weight change over the course of a 4 week study (KYSE520 xenograft from A). **D.** PK/PD relationship of treatment with GDC-1971 in KYSE520 tumor xenografts. Concentration of compound from tumor was assessed by LC/MS and pathway inhibition was determined via pERK AlphaLISA from tumor lysates. Relationship of total GDC-1971 compared to the level of path-

4. GDC-1971 combines with ALK targeted therapies and can reverse resistance in vitro

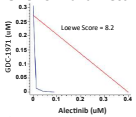
A. Alectinib-resistant ALK-driven cell line model



B. GDC-1971 Potency is Similar in Alectinib-Resistant Model



C. Synergy between GDC-1971 and Alectinib



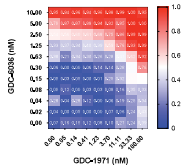
NIH-H3122 (EML4-ALK) NSCLC model was grown in the presence of the ALK inhibitor Crizotinib (1uM), media refreshed biweekly, until cells proliferated in the presence of compound and a Crizotinib-resistant pool was established. In A, B, and C cells were grown in 2D culture conditions in the presence of compound for 4 days. **A.** Crizotinib-resistant model is also resistant to Alectinib. **B.** The potency of GDC-1971 in parental and cells resistant to Alectinib GDC-1971 is similar. **C.** The addition of GDC-1971 to Alectinib reverses resistance to Alectinib alone. Isobolograms were generated by GeneData Screener using Loewe's additivity method.

5. GDC-1971 displays potent synergy in combination with the KRAS G12C Inhibitor GDC-6036 in vitro and in vivo

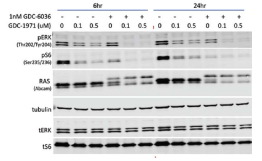
A. GDC-1971 and GDC-6036 are Synergistic in KRAS G12C Mutant Models

Cell line	Positive Bliss
HCC1792	143
SW1573	172
NCI-H358	278
NCI-H23	317
NCI-H2030	385
NCI-H2122	486
HCC4019	698
HCC4017	929

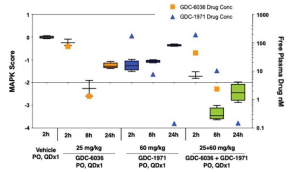
B. Combination Excess Plot of GDC-1971 and GDC-6036 in NCI-H2122



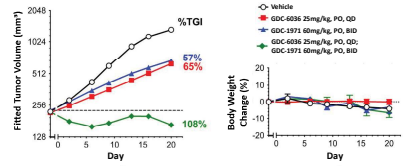
C. MAPK-pathway signaling is suppressed in GDC-1971/GDC-6036 Treated NCI-H2122 Cells



D. Sustained Pathway Inhibition is Observed with in vivo Treatment of GDC-1971 and GDC-6036



E. The Combination of GDC-1971 and GDC-6036 Results in Tumor Regressions in vivo in NCI-H2122 Xenografts



A. Bliss Combination Scores for GDC-1971 in combination with the KRAS G12C inhibitor GDC-6036. Cells were cultured under 3D ULA conditions and treated with a dose matrix of GDC-1971 and GDC-6036 for 7 days. Viability was measured by 3D Celltiter Glo. Bliss score reflects the observed inhibition by the combination treatment over predicted additive inhibition by each agent alone1. **B.** Excess plot of GDC-1971 and GDC-6036 for the KRAS G12C model NCI-H2122. Viability inhibition by a dose matrix of GDC-1971 and GDC-6036 in NCI-H2122 cells grown in 3D condition, incubated with compounds for 7 days. Viability was determined by 3D Celltiter Glo. **C.** Western blot of MAPK signaling in NCI-H2122 cells. Cells were grown in ULA wells for 3D growth and treated with GDC-1971 or GDC-6036 alone and in combination at the doses indicated. After 6 or 24hr of exposure to compound(s) cells were collected and lysates generated. **D.** Potent and sustained inhibition of the MAPK pathway is observed with GDC-1971 and GDC-6036 in combination. Single dose PK/PD analysis performed in NCI-H2122 xenograft tumors. Tumors established in BALB/c Nude mice were dosed with compound and tumors were collected at the time points indicated. Concentration of the compounds in the tumor were determined by LC/MS. The MAPK score, a proxy for MAPK activity, was calculated for each treatment condition as the mean of ddCT (delta cycle Threshold differential with respect to vehicle-treated controls) for the transcripts of the following genes: SPRY2, SPRY4, ETV4, ETV5, DUSP4, DUSP6, CCND1. Analysis was performed using R (ver. 4.0.0). **E.** In vivo response of GDC-1971 and GDC-6036 combination in the NCI-H2122 xenograft model in host BALB/c Nude mice. The combination results in greater tumor regression when compared to either agent dosed alone. The combination is well tolerated with no significant loss of body weight in either of the single agent or in the combination dosing schedules.

Conclusions

- GDC-1971 is a potent allosteric SHP2 inhibitor
- GDC-1971 demonstrates inhibition of the MAPK-pathway in cells, resulting in an anti-proliferative effect
- GDC-1971 achieves significant anti-tumor growth effect as a single-agent in human tumor xenograft models and continuous daily dosing is well tolerated
- The combination of GDC-1971 with Alectinib in cells that have acquired resistance to the ALK inhibitors Crizotinib and Alectinib results in a synergistic anti-proliferative response
- The combination of GDC-1971 with the KRAS G12C inhibitor GDC-6036 results in synergistic effects on cell line viability in KRAS G12C mutant cell line models and drives potent tumor regressions in vivo in the KRAS G12C model NCI-H2122
- The combination trial of GDC-1971 and GDC-6036 is ongoing to assess clinical benefit in KRAS G12C mutant tumors

References

1. Borisy AA, Elliott PJ, Hurst NW, Lee MS, Lehar J, Price ER, et al. Systematic discovery of multicomponent therapeutics. Proc Natl Acad Sci 2003;100:7977–82.