TYRA-200: Potent Against FGFR2 Fusions, Molecular Brake Mutations and Gatekeeper Resistance

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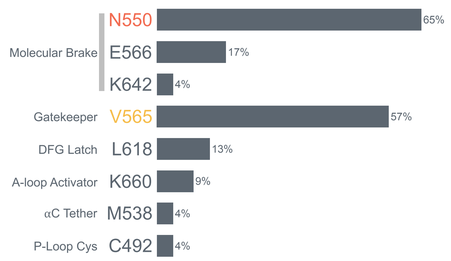
Poster # 47

Background

Approved pan-FGFR (fibroblast growth factor receptor) inhibitors (pemigatinib, infigratinib, and futibatinib) have demonstrated a clinical benefit in metastatic FGFR2-fusion or rearranged intrahepatic cholangiocarcinoma (ICC)[1, 2, 3]. However, inhibition of emerging polyclonal on-target acquired resistance mutations remains a critical unmet need[4, 5, 6].

TYRA-200 is an FGFR1/2/3 inhibitor that was designed to specifically address these clinically observed acquired resistance mutations within the kinase domain of FGFR2. A significant therapeutic benefit may be achieved from this precision approach for FGFR2-driven cancers.

On-Target Acquired Resistance Mutation Frequency

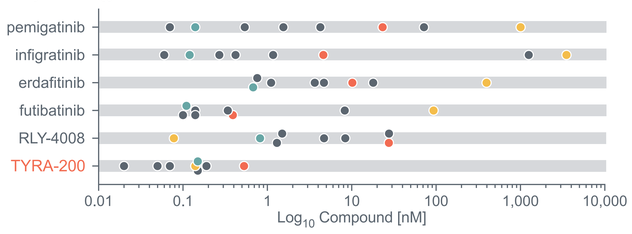


Reversible inhibitor (N=20) = pemigatinib, infigratinib, derazantinib, Debio1347, ATP-competitive inhibitor NOS
Irreversible inhibitor (N=26) = futibatinib
Adapted from data presented by Lipika Goyal at the 32nd EORTC/AACR/NCI Virtual Symposium (Oct 2020, Abs 49).

Results

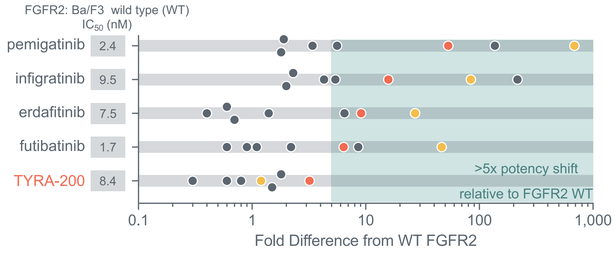
TYRA-200 maintains potency against molecular brake and gatekeeper mutants in enzymatic assays.

Enzymatic IC_{50}: FGFR2 Variants Tested; **WT**, N550D, N550H, N550T, **N550K**, **V565F**, V565L, E566A



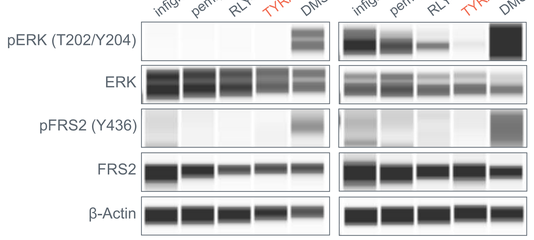
Enzymatic IC_{50} measurements generated at Reaction Biology Corp using Tyra enzymes.
All experiments conducted under identical conditions, tested in duplicate.

TYRA-200 maintains potency for FGFR2 on-target mutations and fusions in Ba/F3 cell lines.



FGFR2 Variants Tested: Clinical fusion 1, Clinical fusion 2, **N550K**, V565F, V565I, K660E, K660N
All experiments tested in identical conditions, tested same day, in duplicate.

TYRA-200 maintains potency against FGFR2 amplifications and molecular brake mutations in cellular assays while inhibiting FGFR2 mediated signaling.



Cells were plated and allowed to settle overnight (~60%-70% confluent) before the addition of vehicle or annotated compound at 50nM for 2 hours. All protein detection was completed via capillary electrophoresis via Simple Western Jess.

Cellular Viability Assays IC_{50} (nM)

futibatinib	infigratinib	pemigatinib	RLY-4008	TYRA-200	
1	3	2	149	4	KG-1 (FGFR1OP2:FGFR1)
6	34	72	14	14	AN3CA ($FGFR2^{K311R,N550K}$)
4	9	8	8	6	SNU-16 (FGFR2amp)
12	10	4	7	7	KATO-III (FGFR2amp)
41	15	13	422	16	RT112/84 (FGFR3:TACC3)
6	9	15	290	5	UM-UC-14 ($FGFR3^{S249C}$)
28	247	129	>3,000	284	MDA-MB-453 ($FGFR4^{V367C}$)

Cell viability was assessed by Cell Titer-Glo 2.0 from Promega. Duration of treatment for IC_{50} generation was cell line dependent and varied from 72-120 hours. IC_{50} values were averaged from three independent experiments.

Results

RNA sequencing identified potential biomarkers for TYRA-200 FGFR activity *in vitro*[7].

SNU-16 cells were treated with vehicle, AZD4547 (100nM), or TYRA-200 (100nM) for 24-hours. Cells were collected for RNA sequencing analysis (heatmap) and confirmatory qRT-PCR analysis (plots).

Conclusions

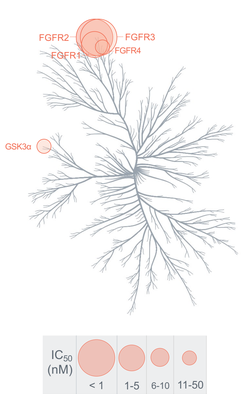
TYRA-200 is currently under development for FGFR2-altered advanced solid tumors, including ICC. Importantly, these data demonstrate that TYRA-200 retains potency across multiple resistance mutations which may emerge during current FGFR therapies, including gatekeeper and molecular brake mutations.

Results

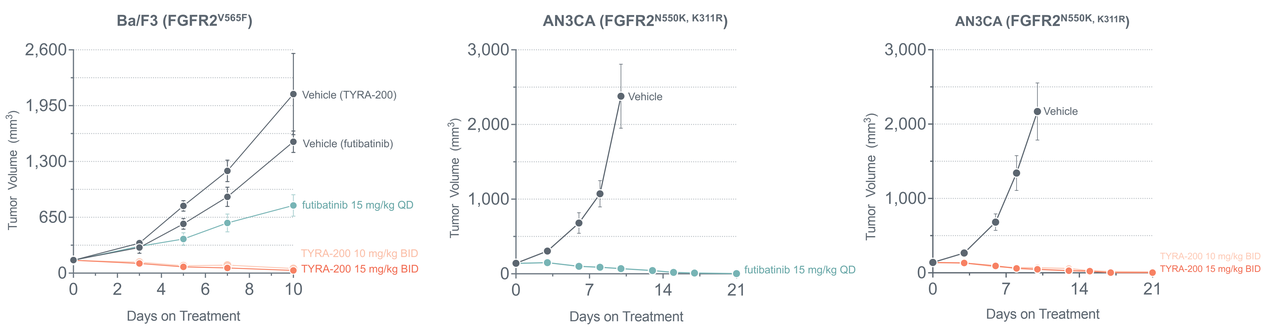
KINOMEscan shows TYRA-200 is highly selectivity for FGFR1/2/3 and spares FGFR4

	TYRA-200	FGFR2 selectivity
FGFR2	0.47	1.0x
FGFR3	0.66	1.4x
FGFR1	1.8	3.8x
FGFR4	30.5	65x
GSK3α	35.6	76x

TYRA-200 was profiled in a scanMAX℠ (KINOMEscan) screen, follow-up IC_{50} data was generated by Reaction Biology Inc.



In vivo tumor efficacy in FGFR2 molecular brake and gatekeeper-mutant cancer xenograft and allograft models.



Mice were inoculated with either Ba/F3 $FGFR2^{V565F}$ (left) or AN3CA (middle & right) cells, then dosed orally with vehicle, TYRA-200, or futibatinib.

References

1. Abou-Alfa GK, Sahai V, Hollebecque A, Vaccaro G, Melisi D, Al-Rajabi R, Paulson AS, Borad MJ, Gallinson D, Murphy AG, Oh DY, Dotan E, Catenacci DV, Van Cutsem E, Ji T, Lihou CF, Zhen H, Féliz L, Vogel A. Pemigatinib for previously treated, locally advanced or metastatic cholangiocarcinoma: a multicentre, open-label, phase 2 study. Lancet Oncol. 2020 May;21(5):671-684.
2. Javle M, Roychowdhury S, Kelley RK, Sadeghi S, Macarulla T, Weiss KH, Waldschmidt DT, Goyal L, Borbath I, El-Khoueiry A, Borad MJ, Yong WP, Philip PA, Bitzer M, Tanasanvimon S, Li A, Pande A, Soifer HS, Shepherd SP, Moran S, Zhu AX, Bekaii-Saab TS, Abou-Alfa GK. Infigratinib (BGJ398) in previously treated patients with advanced or metastatic cholangiocarcinoma with FGFR2 fusions or rearrangements: mature results from a multicentre, open-label, single-arm, phase 2 study. Lancet Gastroenterol Hepatol. 2021 Oct;6(10):803-815.
3. Goyal L, Meric-Bernstam F, Hollebecque A, Valle JW, Morizane C, Karasic TB, Abrams TA, Furuse J, He Y, Soni N, Benhadji KA, Bridgewater JA. FOENIX-CCA2: A phase II, open-label, multicenter study of futibatinib in patients (pts) with intrahepatic cholangiocarcinoma (iCCA) harboring *FGFR2* gene fusions or other rearrangements. Journal of Clinical Oncology 2020 38:15_suppl, 108-108
4. Goyal L, Saha SK, Liu LY, Siravegna G, Leshchiner I, Ahronian LG, Lennerz JK, Vu P, Deshpande V, Kambadakone A, Mussolin B, Reyes S, Henderson L, Sun JE, Van Seventer EE, Gurski JM Jr, Baltschukat S, Schacher-Engstler B, Barys L, Stamm C, Furet P, Ryan DP, Stone JR, Iafrate AJ, Getz G, Porta DG, Tiedt R, Bardelli A, Juric D, Corcoran RB, Bardeesy N, Zhu AX. Polyclonal Secondary *FGFR2* Mutations Drive Acquired Resistance to FGFR Inhibition in Patients with FGFR2 Fusion-Positive Cholangiocarcinoma. Cancer Discov. 2017 Mar;7(3):252-263.
5. Silverman IM, Hollebecque A, Friboulet L, Owens S, Newton RC, Zhen H, Féliz L, Zecchetto C, Melisi D, Burn TC. Clinicogenomic Analysis of *FGFR2*-Rearranged Cholangiocarcinoma Identifies Correlates of Response and Mechanisms of Resistance to Pemigatinib. Cancer Discov. 2021 Feb;11(2):326-339.
6. Krook MA, Lenyo A, Wilberding M, Barker H, Dantuono M, Bailey KM, Chen HZ, Reeser JW, Wing MR, Miya J, Samorodnitsky E, Smith AM, Dao T, Martin DM, Ciombor KK, Hays J, Freud AG, Roychowdhury S. Efficacy of FGFR Inhibitors and Combination Therapies for Acquired Resistance in FGFR2-Fusion Cholangiocarcinoma. Mol Cancer Ther. 2020 Mar;19(3):847-857.
7. Delpuech O, Rooney C, Mooney L, Baker D, Shaw R, Dymond M, Wang D, Zhang P, Cross S, Veldman-Jones M, Wilson J, Davies BR, Dry JR, Kilgour E, Smith PD. Identification of Pharmacodynamic Transcript Biomarkers in Response to FGFR Inhibition by AZD4547. Mol Cancer Ther. 2016 Nov;15(11):2802-2813.

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