Altered response to BET Bromodomain inhibitors JQ1 and I-BET-762 targeting c-Myc in erdafitinib-resistant endometrial carcinoma cell line AN3 CA

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Introduction

- FGFR alterations are common in multiple cancer types, including breast cancer, non-small cell lung cancer and endometrial cancer.
- The pan-FGFR inhibitor erdafitinib is used to treat advanced or metastatic urothelial carcinoma harboring genetic *FGFR2* or *FGFR3* alterations.
- After a good initial response, patients rapidly develop clinical resistance to erdafitinib and have a progression-free survival of only a few months.
- Insight into acquired resistance mechanisms to erdafitinib treatment aids in the development of new therapies.
- Known resistance mechanisms to FGFR inhibitors include acquired mutations in *FGFR*s, alternative signaling via other receptors, i.e., MET, ERBB2/3 or EGFR, activation of internal signaling pathways and epithelial-mesenchymal transition.
- We developed erdafitinib-resistant cell lines *in vitro* and investigated the mechanism of acquired resistance to the drug.

Experimental approach

- Endometrial adenocarcinoma cell line AN3 CA, harboring *FGFR2* gain-of-function mutation N549K, was cultured with gradually increasing concentrations of erdafitinib.
- The concentration was increased when cells were recovered from the previous erdafitinib concentration and were in exponential growth phase.
- The resistant cell lines were compared to the parental cell line on expression of targets, response to small molecule inhibitors and mutation status.

Figure 1. Schematic representation of the in vitro selection of resistant cell lines. *The parental cell line was cultured with starting concentrations of erdafitinib based on drug sensitivity analysis. The concentration erdafitinib with which the cell lines were cultured is indicated.*

Results

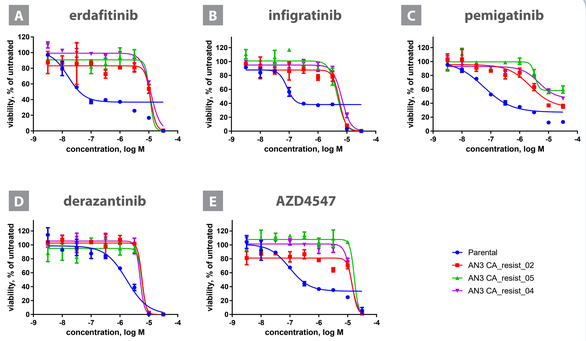

Figure 2. Cell lines selected for resistance against erdafitinib are cross-resistant to other FGFR inhibitors. *Dose-response curves of the AN3 CA parental and resistant lines exposed to pan-FGFR1-4 inhibitor erdafitinib, FGFR1/2/3 inhibitors infigratinib (B), pemigatinib (C), derazantinib (D) and AZD4547 (E). The IC_{50} values are listed in Table 2.*

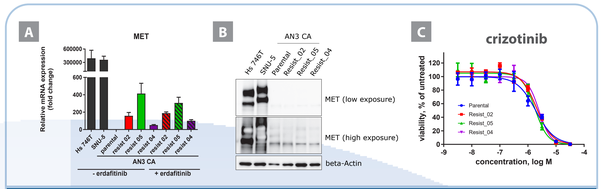

Figure 3. c-MET is not involved in erdafitinib resistance in AN3 CA resistant cell lines. *(A) An increase in c-MET mRNA expression is observed by qPCR analysis. Expression levels were normalized for expression of GAPDH, beta-actin, HPRT1 and RPS18, averaged and related to the parental line. Cell lines Hs 746T and SNU-5 have high expression of c-MET and serve as reference for expression. (B) Increased expression of c-MET is not confirmed in protein expression by immunoblot. (C) In vitro drug sensitivity analysis shows unaltered response of resistant lines to c-MET inhibitor crizotinib when compared to the parental line, indicating c-MET is not the main factor responsible for resistance to erdafitinib.*

Table 1. Mutations in *FGFR1* and *KRAS* were detected by next generation sequencing. *FGFR1* and *KRAS* mutations were not detected in the parental AN3 CA cell line, while an increase in variant frequency was observed in resist_02 and subsequent resistant line resist_05, indicating selection for the mutation occurs.

Gene	Mutation	Variant frequency			
		Parental	Resist_02	Resist_05	Resist_04
FGFR1	M637V	-	18.7	38.0	1.9
KRAS	A146V	-	15.8	39.0	-

Table 2. Genes reported in FGFR inhibitor resistance are not the cause of resistance to erdafitinib in the AN3 CA resistant cell lines. Inhibition of indicated targets by small molecule inhibitors revealed unaltered response (blue) or decreased sensitivity (green) of the resistant lines compared to the parental cell line. IC_{50} values were calculated from dose-response curves.

Compound name	Target(s)	IC_{50} values (nM)			
		Parental	Resist_02	Resist_05	Resist_04
erdafitinib	pan-FGFR (1-4)	16	11433	10789	11595
infigratinib	FGFR1/2/3	84	5520	4663	6640
pemigatinib	FGFR1/2/3	57	2352	3379	4608
derazantinib	FGFR1/2/3	1722	5780	5812	4946
AZD4547	FGFR1/2/3	97	14605	16886	13119
pazopanib	FGFR, VEGFR1/2/3, PDGFR,c-Kit, c-Fms	155	30966	25903	18450
ponatinib	FGFR1, Abl, PDGFRa, VEGFR2, Src	24	800	937	1120
nintedanib	VEGFR1/2/3, FGFR1/2/3, PDGFRa/ß	237	3529	3806	ND
sunitinib	PDGF-Rs, VEGFRs, c-KIT, RET, CD114, CD135	939	4080	5017	3442
osimertinib	EGFR	2849	2891	2929	3014
lapatinib	EGFR, ERBB2	14201	15803	11989	14990
neratinib	EGFR, ERBB2	>3160	>3160	2800	>3160
tucatinib	ERBB2	24686	16384	20529	29794
crizotinib	MET, ALK	1903	2039	1782	2472
cabozantinib	VEGFR2, c-Met, Ret, Kit, Flt-1/3/4, Tie2, AXL	3088	>31600	>31600	19570
trametinib	MEK	50	6631	23751	286
PD 0325901	MEK	1146	1457	2619	710
metformin	AMPK activator (mTORC inhibition)	>31600	>31600	>31600	>31600
Bay-293	KRAS-SOS1 interaction inhibitor	2654	2485	2294	2738
ipatasertib	AKT	114	480	634	780
MK-2206	AKT	540	8390	6607	4693
dactolisib	PI3K/mTOR	51	112	102	145
everolimus	mTOR	59	138	53	208
temsirolimus	mTOR	830	205	1006	610

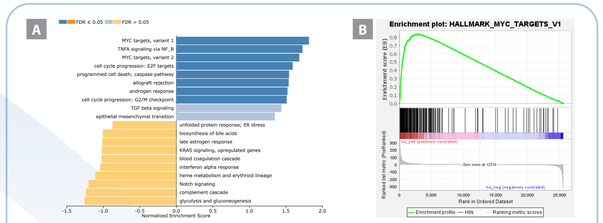

Figure 4. Altered expression of Myc target genes revealed by RNA sequencing. *(A) The parental and resist_05 cell line were subjected to RNA sequencing and subsequent gene set enrichment analysis was performed to detect enriched gene sets. (B) Enrichment plot for Myc target gene set V1.*

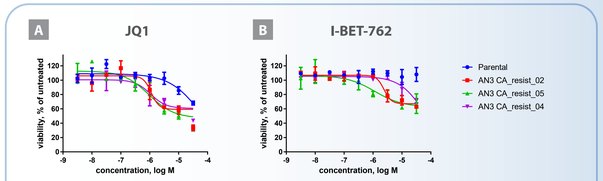

Figure 5. In vitro drug sensitivity analysis confirms that the resistant cell lines have increased sensitivity to c-Myc targeting. *BET Bromodomain inhibitors JQ1 and I-BET-762 were used to indirectly target c-Myc. Dose-response curves of the AN3 CA parental and resistant lines exposed to (A) JQ1 and (B) I-BET-762.*

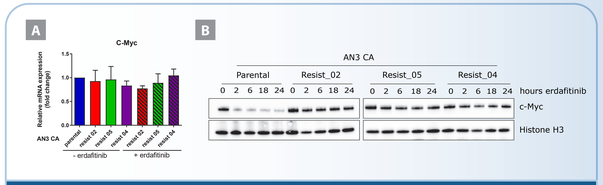

Figure 6. Alterations in c-Myc stability are seen in the resistant cell lines. *(A) No alterations in c-Myc expression are observed in mRNA expression as determined by qPCR analysis. Expression levels were normalized for expression of GAPDH, beta-actin, HPRT1 and RPS18, averaged and related to the parental line. (B) Immunoblot showing a time course of erdafitinib treatment on parental and resistant cell lines reveals altered stability of c-Myc in resistant cell lines.*

Conclusions

- Erdafitinib-resistant AN3 CA cell lines were generated by gradually increasing the concentration of erdafitinib during cell culture.
- Erdafitinib-resistant lines showed cross-resistance to other FGFR inhibitors.
- Multiple factors were identified which may contribute to the resistance to erdafitinib:
  - Missense mutations in *FGFR1* and *KRAS* were found in increasing frequency after selection on higher concentrations of erdafitinib.
  - c-Myc was detected to have an altered stability in erdafitinib-resistant lines resulting in differential expression of c-Myc target genes.
- BET Bromodomain inhibitors, that indirectly target c-Myc, are of potential interest as therapeutic agents to overcome resistance to erdafitinib.

References: Lau *et. al.* (2019) Cancer Drug Resistance 2:568-579; Zhou *et. al.* (2020) Journal of Cancer 11:2000-2007; Yue *et. al.* (2021) Journal of Hematology & Oncology 14:23