X-MAN™ Isogenic DualXeno™ models with KRAS Mutation Predicts the Effect of Anti-EGFR Agents

Abstract # 3136

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Introduction

We have previously reported using cell lines generated with Horizon Discovery's rAAV-based GENESIS™ gene editing platform to establish a comprehensive list of isogenic cancer models for *in vivo* compound screening, with mutations in a wide variety of genes including KRAS, PIK3CA, PTEN, IDH1 and IDH2, and p53. These isogenic tumor models comprise pairs of cell lines which share the same genetic background, differing only by the mutation of interest, and therefore allow definitive studies of specific genetic variances to be performed.

In the current study we developed a DualXeno™ method where isogenic pairs of a colorectal cell line, one of each pair with a KRAS mutation, were inoculated simultaneously in the two flanks of the same mouse. The tumors were then treated with EGFR targeted therapeutics to address the question of resistant phenotypes elicited by different KRAS mutations. This design allows direct comparison of wild type and mutant isogenic pairs for treatment responses that are associated with the defined genetic variations. Our results demonstrated that tumors harboring the G12V mutation were resistant to both Cetuximab and Erlotinib treatment, while tumors harboring the G13D mutation remained sensitive to both agents. This is in consistent with clinical findings (De Roock, et al. JAMA 2010, 304(16), pp 1812), and our own findings with PDX mouse clinical trials in colon cancer, suggesting that the KRAS G13D mutation may establish a different signaling network to other KRAS mutations, and that colon cancer patients with the mutation should not be excluded from the EGFR targeted therapies.

Materials and Methods

Animals

Nu/Nu male mice purchased from HFK Bioscience Co., Ltd. (Beijing, China) were used for DualXeno studies using series of SW48 colorectal carcinoma isogenic lines (Horizon Discovery, UK).

Tumor inoculation

Each mouse was subcutaneously injected with 1 × 10^7 SW48 tumor cells in 0.1 ml PBS in the left and right front flank for tumor development.

Data collection

Tumor volume was measured at 3 day intervals until mean value reached 2000mm^3 where possible.

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Isogenic lines tested *in vivo*

Cell line	Horizon Catalogue Number	Genotype	Catalogue Description
SW48	HD PAR-006	Parental	
	HD 103-002	KRAS (G13D/+)	Heterozygous knock-in of KRAS activating mutation (G13D)
	HD 103-007	KRAS (G12V/+)	Heterozygous knock-in of KRAS activating mutation (G12V)

Groups and Treatments

Group	Animal No.	Location	Tumor	Cell No.	Treatment
1	15	Left flank	SW48-parental HD PAR-006	$1x10^7$	Vehicle (5 mice), Cetuximab (5 mice) Erlotinib (5 mice)
		Right flank	HD 103-002 KRAS (G13D/+)	$1x10^7$	
2	15	Left flank	SW48-parental HD PAR-006	$1x10^7$	Vehicle (5 mice) Cetuximab (5 mice) Erlotinib (5 mice)
		Right flank	HD 103-007 KRAS (G12V/+)	$1x10^7$	

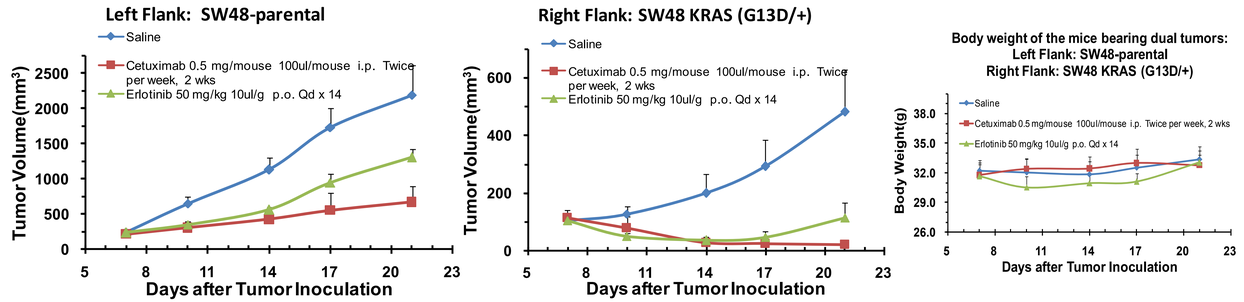
Details for treatment

Treatment	Dose (mg/kg)	Dosing Route	Schedule
Vehicle (saline)	-	-	-
Cetuximab	0.5 mg / mouse / treatment	*i.p.*	Twice per week, 2 wks
Erlotinib	50 mg/kg	*p.o.*	Qd x 14

References

1. DeRook W, Jonker DJ, Di Nicolantonio F, et al. Journal of American Medical Association. 2010, 304 (16):1812-20
2. Jane A. Plumb, Gordon Strathdee, Julieann Sludden, et al. Cancer Research. 2000, 60; 6039-44

SW48 DualXeno Model: SW48-parental and SW48 KRAS (G13D/+)



SW48 DualXeno Model: SW48-parental and SW48 KRAS (G12V/+)

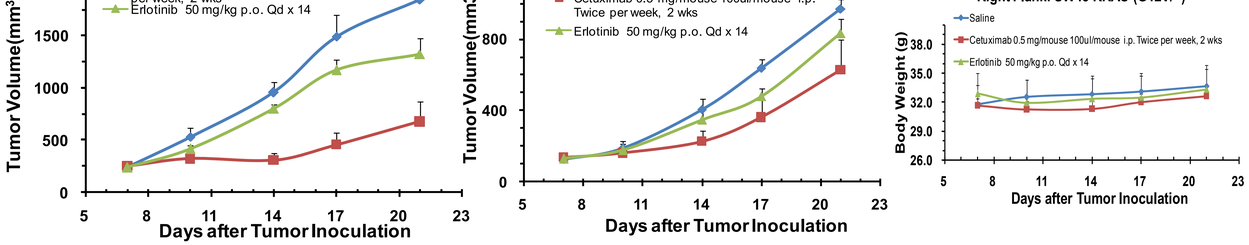


Table 1. Antitumor Activity on Tumor Volume of Erlotinib in the Treatment of SW48-parental, SW48 KRAS (G13D/+) and SW48 KRAS (G12V/+) Isogenic DualXeno™ models

Erlotinib Treatment (50 mg/kg, p.o., Qd x 14)	T/C on Day21	TGI on Day21
SW48 parental line	59%~72%	28%~41%
SW48 KRAS(G13D/+)	24%	76%
SW48 KRAS(G12V/+)	86%	14%

Table 2. Antitumor Activity on Tumor Volume of Cetuximab in the Treatment of SW48-parental, SW48 KRAS (G13D/+) and SW48 KRAS (G12V/+) Isogenic DualXeno™ models

Cetuximab Treatment (0.5 mg/mouse, i.p. BIW x 2 wks)	T/C on Day21	TGI on Day21
SW48 parental line	30%~37%	63%~70%
SW48 KRAS(G13D/+)	4%	96%
SW48 KRAS(G12V/+)	65%	35%

Conclusions

➢SW48 KRAS (G12V/+) is resistant to Erlotinib and Cetuximab compared to the parental line: in the SW48 parental line, Erlotinib treatment resulted in TGI=28~41%, and Cetuximab treatment resulted in TGI=63~70%; in SW48 KRAS (G12V/+), Erlotinib treatment resulted in TGI=14%, and Cetuximab treatment resulted in TGI=35%.

➢SW48 KRAS (G13D/+) remains sensitive to Erlotinib and Cetuximab compared to the parental line: in the SW48 parental line, Erlotinib treatment resulted in TGI=28~41%, and Cetuximab treatment resulted in TGI=63~70%; in SW48 KRAS (G13D/+), Erlotinib treatment resulted in TGI=76%, and Cetuximab treatment resulted in TGI=96%.

➢Our data support the clinical finding (Wendy De Roock, et al. JAMA 2010, 304(16), pp 1812) that KRAS mutation G13D is quite different from other KRAS mutations, and cells carrying the mutation can remain sensitive to EGFR targeting agents such as Cetuximab and Erlotinib.

➢*In vivo* isogenic models harboring KRAS mutations are useful tools to screen for next generation agents targeting resistance mechanisms.