Drug Resistant Murine Tumor Models Facilitate the Development of Next Generation Anticancer Therapeutics

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Introduction

Despite the development of promising new anticancer drugs, all cancer therapeutics, including conventional cytotoxic chemotherapy or the latest targeted agents, are limited by the emergence of drug resistance. Resistance may be divided into two broad categories: intrinsic or *de novo* and acquired, involving different mechanisms. Some patients are insensitive to standard of care (SoC) agents, hence are considered *de novo* resistant, while others initially respond, but eventually relapse with resistant tumors that are no longer sensitive to treatment. Even in the case of the newly developed immunotherapies, which promise to have a long lasting tumor inhibitory effect, a large percentage of patients still does not respond. To overcome drug resistance to the existing standard of care agents, effective new strategies, such as a next-generation of selective inhibitors, rational drug combinations, and new drug delivery or formulation designs, are developed and are under active preclinical and clinical investigation. To enable a more informative selection of resistant models for *in vivo* preclinical drug evaluation, here we screened more than 200 CrownBio validated cell line-derived xenograft (CDX) and syngeneic tumor models for the identification of those animal models, which are intrinsically resistant to either chemotherapy, targeted therapy, or immunotherapy. We also validated a panel of acquired drug resistant models, covering, among others, the resistance to Doxorubicin, Imatinib, Elotinib, Quizartinib, Crizotinib or Androgen ablation (castration). Possible mechanisms of resistance were discussed for some models. In conclusion, our models provide a valuable platform for the development of new strategies to overcome the drug resistance.

Models Resistant to Chemotherapy

Cancer Type	Cell Line	SOC	Dosing Schedule	T/C% at the end of treatment	p value
Leukemia	THP-1	decitabine	0.2mg/kg, s.c., -TIW x 2	80%	0.107
		daunorbicin	3mg/kg, i.v., Q4D x 4	104%	0.871
		Ara-C	40-70mg/kg, i.p., qd X 3 wks(in rat)	90%	0.642
Gastric Cancer	SNU-5	5-FU	60mg/kg, i.p., Q7D x 3	102%	0.925
	SNU-16	5-FU	100mg/kg, i.p., Q7D x 1 75mg/kg, i.p., Q7D x 3	72%	0.158
Lung Cancer	NCI-H1650	cisplatin	4mg/kg, i.v., Q7D x 3	85%	0.357
	H1975	cisplatin	5mg/kg, i.p., Q7D x 3	82%	0.247
	H69AR (acquired resistance)	doxorubicin	4mg/kg, i.v., Q4D x 4	74%	0.024
Breast Cancer	MX-1	docetaxel	6mg/kg, i.v., Q7D x 3	78%	0.290
		doxorubicin	5mg/kg, i.v., Q4D x 3	112%	0.554

Table 1. Models of Intrinsic Resistance to Chemotherapy

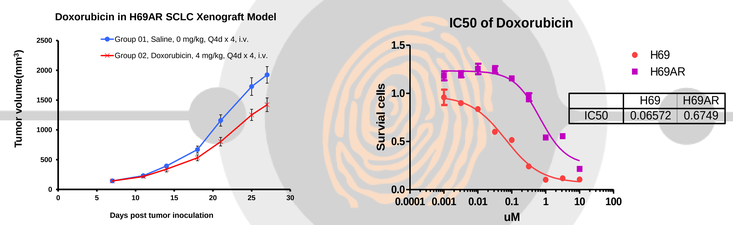

Figure 1. Acquired Resistance to Doxorubicin in the SCLC H69AR Model. H69-AR was sourced from ATCC and was established from NCI-H69 cells (ATCC HTB-119) that were grown in the presence of increasing concentrations of doxorubicin over a total of 14 months. Reduced susceptibility to drug-induced DNA damage[1] is one of the multiple mechanisms contribute to drug resistance in this SCLC model.

Models Resistant to Targeted Therapy

SoC Drug	Highly Responsive Cell Lines	Partially Responsive Cell Lines	Non-Responsive Cell Lines
Bevacizumab	H358, SK-OV-3, MKN45, A673	COLO 205, HCT116, A549, NCI-H1299,	LLC,KP4, CT26, HT29, Panc-1, MCF-7
Cetuximab	H1975, H358, H292, MiaPaCa-2, SW48 (Kras WT), SW48 (Kras G13D mutant)	Colo205, SNU-5	EBC-1, HCT116, MKN45, NCI-H1993, NCI-H820, SW48(Kras G12V mutant)
Trastuzumab	BT-474, NCI-N87, SK-OV-3	YCC-2	
Rituximab	RL, DOHH-2	Raji,WSU-DLCL2	Daudi
Erlotinib	NCI-H292, NCI-H1650, HCC827, HCC827-ER1	A431, Hep3B, HepG2	EBC-1, NCI-H1993, NCI-H1975
Sorafenib	HUH-7, PLC/PRF/5, MHCC97H (orth)	Hep3B, HepG2, MHCC97H(s.c.)	Caki-1
Afatinib	A431, NCI-N87, H1975, HCC827		EBC-1, NCI-H1993, MDA-MB-453
Crizotinib	MKN45, U87MG, EBC-1, NCI-H820, NCI-H2228	NCI-H1993,KP4, KARPAS 299, SNU-2535	NCI-H292
Ibrutinib	Pfeiffer	DOHH2	
Imatinib	K562	Kasumi-1	K562G (acquired resistance)
Quizartinib	MV4-11, MOLM-13		MV4-11-QR (acquired resistance)

Table 2. Summary of Responses to Targeted Agents

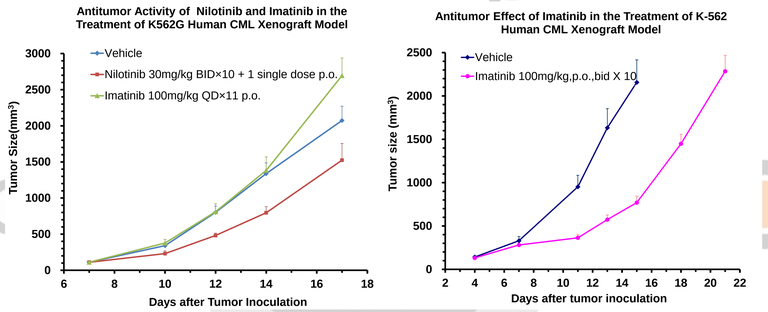

Figure 2. Acquired Resistance to Imatinib in the K562G Model. Resistance was induced by *in vitro* exposure of K562 cells to escalating concentration of Imatinib. Increased expression of BCR/ABL and mdr1/P2gp, BCR/ABL gene fusion, and increased activity of BCR/ABL[3] underline possible mechanisms of resistance.

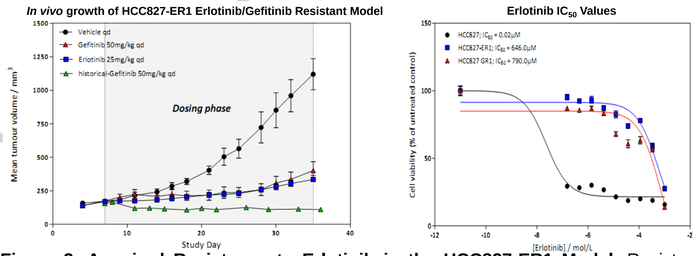

Figure 3. Acquired Resistance to Erlotinib in the HCC827-ER1 Model. Resistance was induced by repeated *in vitro* exposure to escalating concentrations of Erlotinib. Possible mechanism of resistance include: increased c-Met copy number; increased Axl expression level; exon19 (E746-A750) deletion confirmed in all samples. No exon20 (T790M) mutation detected.

Models Resistant to Targeted Therapy (Continued)

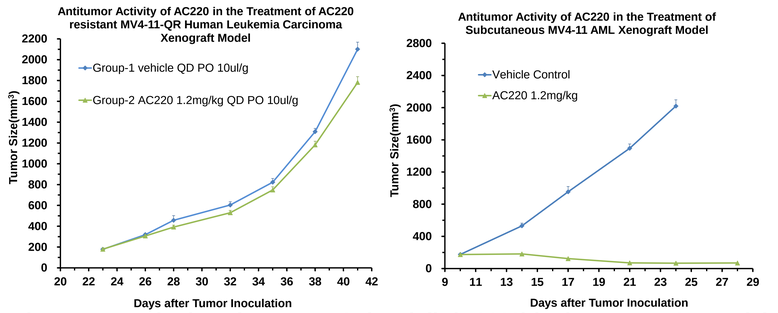

Figure 4. Acquired Resistance to Quizartinib (AC220) in the MV4-11-QR Model. The model was passaged *in vivo* under AC220 pressure for more than one year. In the clinic resistance to quizartinib depends on secondary mutations at 3 residues in the FLT3-ITD kinase domain: the "gatekeeper" residue F691, the activation loop (AL) residues D835, and Y842[4]. Mechanisms of Quizartinib resistance in MV4-11-QR are currently under investigation.

Responses to Immune-Checkpoint Inhibitors in Syngegeic Models

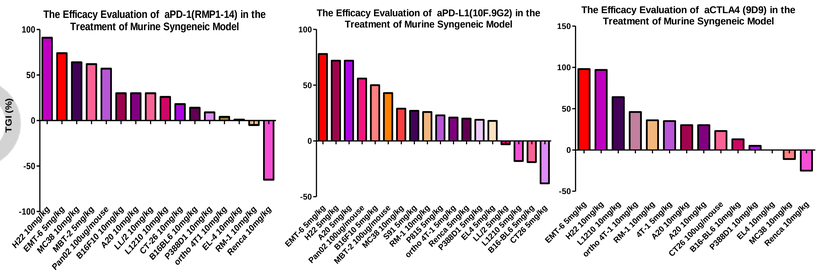

Figure 5. Evaluation of aPD-1 (RMP1-14), aPD-L1 (10F.9G2) and aCTLA4 (9D9) Antibodies Efficacy in the Treatment of Murine Syngeneic Models

References

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2. Mésange P.et al. Oncotarget,2014,5: 4709-4721.
3. Qi J, et al. Chin J Hematol ,June 2004 ,Vol 25 ,No. 6.
4. Catherine C Smith.et al. *Blood* (ASH Annual Meeting Abstracts) 2012 120: Abstract 141.

Additional data are available upon request. Please contact the corresponding author Dr. Qian Shi, shiqian@crownbio.com or log on to http://XenoBase.Crownbio.com for detailed model information.

Summary

- We established the responses to SoC/reference drugs, targeted therapy and immunotherapy in a set of models of *de novo* and acquired to facilitate the study design or evaluation of the next generation of drugs;
- Some of the mechanisms of drug resistance need to be further investigated.