Induction of Resistances to Crizotinib in NSCLC Patient-Derived Xenograft Models Upon Treatment

Poster # 394

Jianyun Deng, Zhun Wang, Jie Cai, Sheng Guo, Jean-Pierre Wery, Henry Q.X. Li*

Crown Bioscience, Inc., 3375 Scott Blvd, suite 108, Santa Clara, CA 95054, USA. *Presenting Author henryli@crownbio.com

Introduction

Anaplastic lymphoma kinase (ALK) is a receptor tyrosine kinase, and its mutation, overexpression, and gene fusion have been associated with various forms of cancer. EML4-ALK fusion has been confirmed to be the oncogenic driver in a small subset of lung cancers (~4% of NSCLC)[1]. ALK rapidly became an excellent drug target, leading to the development of crizotinib, an ALK tyrosine kinase inhibitor as the first effective therapy for this subset of NSCLC[2,3]. However, like most cancer therapies so far, continuous crizotinib treatment over a prolonged period of time leads to the development of drug resistance, rendering the treatment ineffective[4]. Understanding the mechanisms causing drug resistance can potentially facilitate overcoming the problem and extend patients' life[5,6]. However, lack of experimental model hinders research in this area.

Abstract

We recently established a large collection of lung cancer patient-derived xenografts (~350 PDXs)[7], and we screened some of them for the presence of ALK gene fusion (by RT-PCR and RNAseq). The ALK fusion-positive models were tested for sensitivity to crizotinib. We kept these PDXs under crizotinib treatment pressure *in vivo* in order to induce acquired resistance, as seen in the clinic. The resistant models were genomically profiled to identify the changes that could potentially be responsible for the emergence of crizotinib resistance.

We identified the NSCLC-ADC LU1656 model as a candidate for our study since it displays EML4-ALK fusion and elevated ALK expression (type-1). This model initially responds well to crizotinib *in vivo*. Continuous treatment eventually leads to the development of resistance to crizotinib, a pattern already observed in patients undergoing the same treatment. We called the resistant model LU2445. We performed transcriptome sequencing of the tumor samples from the parental sensitive (LU1656) and the selected resistant (LU2445) models. So far, we found both models expressed similarly high levels ALK, but we could no detect additional mutations in ALK gene. Further data analysis is ongoing.

Results

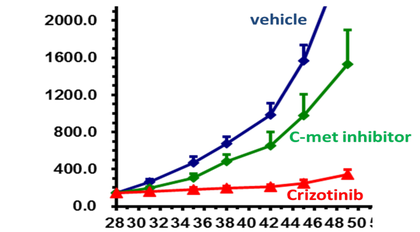

Figure 1: The LU1656 NSCLC PDX model is sensitive to crizotinib but not to *MET* inhibitors

ID	Ethnicity	Gender	Hospital pathology	Pathology QC
LU1656	Asian	Female	NSCLC, Carcinosarcoma; CK (+), Vim (+), Act(-), NSE(-), Syn (-), CD56 (±), Chr-A (-).	Moderately differentiated squamous cell carcinoma (P5).
LU2445	Asian	Female	Carcinosarcoma	Poorly differentiated tumor, giant cells, spindle cells and clear cells observed.

Table 1: Summary of patient and PDX model information

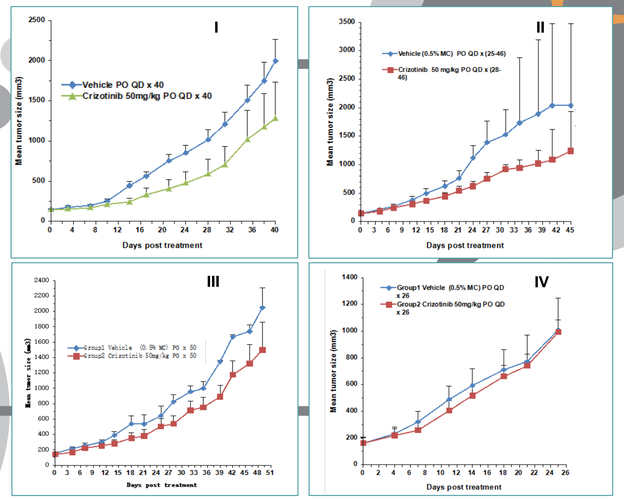

Figure 2: Generation of crizotinib resistant model LU2445 by repetitive exposure of LU1656 sensitive model

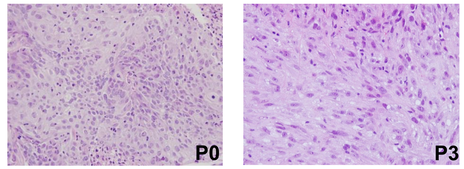

Figure 3: H&E Stain of LU1656

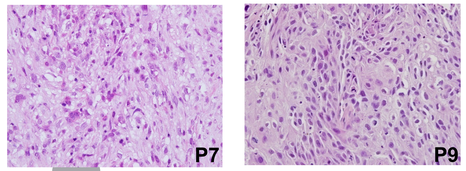

Figure 4: H&E Stain of LU2445

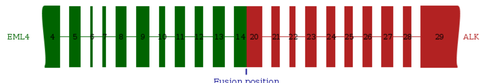

Figure 5: EML4-ALK fusion in LU1656

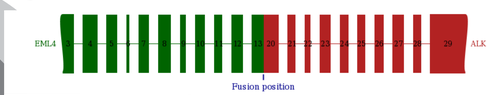

Figure 6: EML4-ALK fusion in LU2445

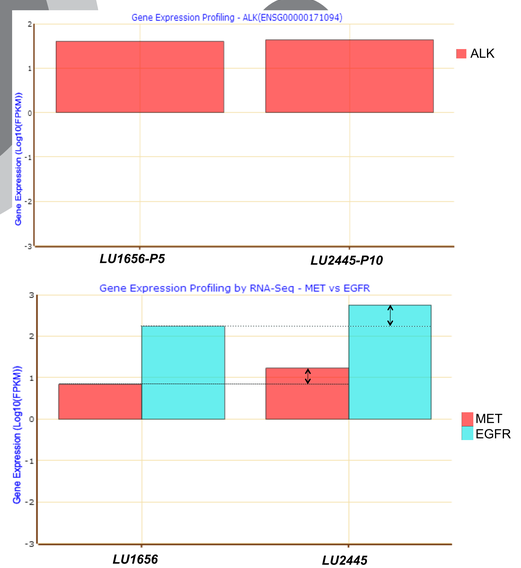

Figure 7: ALK, MET and EGFR expression in LU1656 *vs.* LU2445 NSCLC PDX models

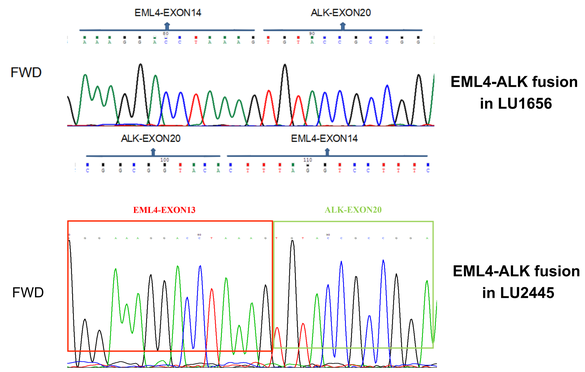

Figure 8. EML4-ALK fusion in LU1656 *vs.* LU2445

Conclusions

1. The NSCLC PDX model LU1656 carries a EML4-ALK fusion;
2. LU1656 is sensitive to crizotinib;
3. LU1656 has no c-MET activation, mutation or amplification, and is insensitive to c-MET inhibitors;
4. LU1656 partially responds to EGFR inhibitors (Erbitux®, erlotinib);
5. Prolonged exposure to crizotinib *in vivo* causes development of resistance in the LU1656 model;
6. No further ALK mutations or change in the level of expression were identified in the resistant model. The molecular mechanism underlying the observed resistance is still to be identified.

References

1. Koivunen, J.P., et al. EML4–ALK fusion gene and efficacy of an ALK kinase inhibitor in lung cancer. Clinical cancer research: an official journal of the American Association for Cancer Research 14, 4275–4283 (2008).
2. Kwak, E.L., et al. Anaplastic lymphoma kinase inhibition in non–smallcell lung cancer. N Engl J Med 363, 1693–1703 (2010).
3. Takeuchi, K., et al. RET, ROS1 and ALK fusions in lung cancer. Nature medicine 18, 378–381 (2012).
4. Katayama, R., et al. Mechanisms of acquired crizotinib resistance in ALK–rearranged lung Cancers. Science translational medicine 4, 120ra117 (2012).
5. Katayama, R., et al. Two novel ALK mutations mediate acquired resistance to the nextgeneration ALK inhibitor alectinib. Clinical cancer research : an official journal of the American Association for Cancer Research 20, 5686–5696 (2014).
6. Friboulet, L., et al. The ALK inhibitor ceritinib overcomes crizotinib resistance in nonsmall cell lung cancer. Cancer discovery 4, 662–673 (2014).
7. Yang, M., et al. Overcoming erlotinib resistance with tailored treatment regimen in patientderived xenografts from naive Asian NSCLC patients. Int J Cancer 132, E74–84 (2013).

Contact information:
HenryLi@crownbio.com