Peer Review File

Article information: https://dx.doi.org/10.21037/jtd-23-1964

Reviewer A

This is an interesting proof-or-principle work demonstrating the potential clinical utility of organoids in the management of ALK+ NSCLC failing TKI. The following points could help improve the manuscript further:

1. lines 62-63: "alectinib is currently the preferred first-line treatment" -> one preferred first-line treatment (brigatinib and lorlatinib are equally preferred; if there are any availability/reimbursement problems in your country, please clarify this).
Reply 1: Thank you for your reminder. In China, among the recommended drugs for first-line treatment of ALK-positive patients, alectinib is recommended as the first choice. We have modified our text as advised (see Page 6, line 75-76).
Changes in the text: L75-76: In China, alectinib is the preferred recommendation for first-line treatment of patients with ALK-positive advanced NSCLC.

2. line 139: EML4-ALK (n=5, 14%): could you please specify the exons? (or explain that these are unknown and why)
Reply 2: Thank you for your reminder. We have modified our text as advised (see Page 11, line 190-192).
Changes in the text: L190-192: EML4 exon 18-ALK exon 20 (n = 2; 6%), EML4 exon 19-ALK exon 20 (n = 1; 3%), EML4 exon 14-ALK exon 21 (n = 1; 3%), EML4 exon 21-ALK exon 20 (n = 1; 3%)

3. could you please specify by which method the ALK was detected at initial diagnosis?
Reply 3: Thank you for your reminder. We have modified our text as advised (see Page 7, line 96-97).
Changes in the text: L96-97: Tissue samples or blood samples were taken for genetic testing and were positive for the ALK fusion gene positivity indicated by NGS.

4. standard treatment after alectinib is actually the 3G inhibitor lorlatinib. Could you please explain why ceritinib (which is less active, e.g. according to ref. 13 of the paper) was chosen? (e.g. lorlatinib was not available / reimbursed, patients choice in oder to avoid the side effects of lorlatinib etc)?
Reply 4: At the time of the patient's resistance to alectinib, the lorlatinib was not included in the coverage list of health insurance policies in China, therefore patients cannot be reimbursed(see Page 12, line 206-207).
Changes in the text: L206-207: This approach was taken because the third-generation drug lorlatinib was not covered by health insurance reimbursement policies at the time.

5. In the main text the authors mention bugitinib, but in the x-axis of Figure 5 brigatinib. One of the two terms should be used consistently throughout the work, and an explanatory note that this is the same drug could also help.
Reply 5: Thank you for your reminder. The drug referred to as "bugitinib" in the text is, in fact, a misnomer; the correct spelling of the medication is "brigatinib." These two terms denote the same therapeutic agent, and any occurrence of "bugitinib" should be amended to "brigatinib" to ensure proper

identification and reference. We were really sorry for our careless mistakes(see Page3, line 44; Page13, line 235; Page16, line 304).
Changes in the text: L44: bugitinib → brigatinib, L235: bugitinib → brigatinib, L304: bugitinib → brigatinib

6. A particular strength of the work is the use of organoids to guide subsequent management for ALK+ NSCLC failing TKI. The authors could provide some technical details about how they did the organoid culture from ALK+ tumors in order to complete documentation of their work and allow other investogators to follow in a similar direction.
Reply 6: Thank you for your suggestion. We added some technical details(see Page8-10, line 123-155).
Changes in the text: L123-155:
Culture of patient-derived organoid (PDO)
Taking a sample of the patient's tumor tissue. Five to six tumour fragments were generated using ophthalmic scissors, approximately 1-3mm, and separated into cold Hank's Balanced Salt Solution (HBSS) with antibiotics (Lonza, Basel, Switzerland) and transported to the laboratory on ice within 1 h of resection from patients. After washing thrice with cold HBSS with antibiotics and sectioning with sterile blades, the samples were incubated with 0.001% DNase (Sigma-Aldrich, MO, USA), 1 mg/mL collagenase/dispase (Roche, IN, USA), 200 U/mL penicillin, 200 mg/mL streptomycin, and 0.5 mg/mL amphotericin B (2% antibiotics, Sigma) in DMEM/F12 medium (Lonza) at 37 °C for 3 h with gentle agitation and intermittent resuspension. Thereafter, the digested tissue suspension was repeatedly triturated via pipetting and passed through a 70-μm filter. The strained suspension was centrifuged at 112 × g for 3 min, and red blood cells were lysed using lysis buffer (00443357, Invitrogen eBioscience) for 5 min. The pellet was resuspended in 100 μL MBM (serum-free medium DMEM/F12; Lonza) supplemented with 20 ng/mL bFGF (Invitrogen, CA, USA), 50 ng/mL human EGF (Invitrogen), N2 (Invitrogen), B27 (Invitrogen), 10 μM ROCK-inhibitor (Enzo Life Sciences, NY, USA), and 1% penicillin-streptomycin (Gibco, OK, USA). Thereafter, 200 μL Matrigel (Corning, NY, USA) was added to 100 μL of the cell suspension for establishing organoids, and the resulting suspension was allowed to solidify on pre-warmed 6-well culture plates (Corning, NY, USA) at 37 °C for 30 min. After gelation, 3 mL MBM was added to the well. The medium was changed every 4 d. Organoids were passaged every 2 weeks with the ratio of 1:2 or 1:3. The procedure of organoid passaging was modified from the methods that already described. Briefly, organoids were harvested by incubating with cold PBS for 1 hour at 4℃ and dissociated using 1× TrypLe Express Enzyme (Gibco), the dissociated organoids were mixed in MBM+Matrigel (1:3 ratio) and reseed in patri dish, then added the medium after gelling. Early passage organoids (<3 passages) were frozen in liquid nitrogen.

Organoid drug response assay
Organoids were harvested using 1× TrypLe Express Enzyme (Gibco) and dissociated to small clusters. Between 100 and 500 clusters were plated in 40-μl 5% Matrigel/organoid culture medium in a 384-well plate (Corning) in triplicate. Clear bottom 384-well plates were coated with 10-μl collagen (Thermo Fisher) prior to plating cells. 48 hours after plating, a concentration dilution series of each compound (10 μM, 2.5μM, 0.625μM, 0.156μM, 0.039μM, 0.0098 μM) was dispensed using liquid-handling robotics, and cell viability was assayed using CellTiter-Glo (Promega) after 4 d of drug incubation. Results were

normalized to vehicle controls. Data analyzed using GraphPad Prism 7.0 software, and the values of IC50, were calculated by applying nonlinear regression (curve fit).

Reviewer B

This manuscript contains a few language/formatting issues. The suggested changes have been marked below. The manuscript could be considered for publication after addressing these issues.

Suggested changes:

L32: Moreover, Next-generation sequencing (NGS) data → Moreover, next-generation sequencing (NGS) data (lowercase for "next")

Reply: Thank you for your reminder. L44: Next-generation → next-generation.

L58 (highlight box/point 3): In this study, a new stretagy → In this study, a new strategy (spelling mistake)

Reply: Thank you for your reminder. L58: stretagy → strategy.

L93: please define TNM in its first instance

Reply: Thank you for your reminder. L94-95: the Tumour, Nodes, and Metastasis (TNM) staging system.

L114: add a full stop at the end of the sentence

Reply: Thank you for your reminder. L117.