Utilizing NSCLC PDXs derived from patients on osimertinib (AZD9291) clinical trials to further refine therapeutic strategies

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Background: Osimertinib (AZD9291)is a mutant-selective EGFR tyrosine kinase inhibitor (TKI) effective against EGFR activating and the T790M acquired resistance mutations.Osimertinib has been approved by the US FDA for patients with EGFR T790M positive NSCLC with resistance to first line EGFR TKIs. However, as acquired resistance to osimertinib is now emerging, we aimed to develop PDXs from patients with: a) acquired resistance to first-line EGFR TKIs prior to enrolling on osimertinib trials and b) post-osimertinib resistance.
Methods: Pre- and post-osimertinib tumor biopsies were implanted into the flank or sub-renal capsule of NSG mice. Successfully established PDXs were expanded and confirmed by ddPCR and NGS to maintain molecular fidelity to the original patient tumor. The osimertinib efficacy in a subset of PDX models was tested and compared to clinical osimertinib response in the patients from whom the PDXs were derived. Acquired osimertinib resistance models were further treated with a panel of novel targeted agent combinations, matching the potential mechanism of osimertinib resistance.
Results: 46 patients underwent a pre-osimertinib or post-osimertinib biopsy. 26 biopsies were from the AURA trial for patients with acquired resistance to first-line EGFR TKIs; 5 biopsies were from the TATTON trial for patients without a T790M mutation; and 7 biopsies were from patients with acquired resistance to osimertinib. A platform of 16 PDX models have been successfully developed and shown to exhibit diverse mechanisms of TKI resistance as confirmed by NGS. These models include: 6 with EGFR T790M; 4 with EGFR non-T790M resistance to erlotinib; and 4 with acquired resistance to osimertinib. In the drug efficacy studies, PDX sensitivity to osimertinib is shown to be comparable to the corresponding patient's clinical response. In the DFCI-243 model (patient: EGFR T790M+, PR 8.9 months), tumors showed regression during the dosing phase and rapidly regrew upon cessation of osimertinib treatment. In contrast, in the DFCI-217 model (patient EGFR T790M+, PR >24 months), tumors showed sustained regression even after osimertinib treatment cessation. In the DFCI-284, a potential model of primary AZD9291 resistance tumors showed no regressions with osimertinib treatment. PDX models have also been used to refine treatment approaches for acquired resistance to osimertinib. DFCI-306 model established from a patient who developed an acquired BRAF mutation while on osimertinib has been shown to respond to either selumetinib or the selumetinib/osimertinib combination.
Conclusion: We have developed a platform of NSCLC PDXs from patients with acquired resistance to first-line EGFR TKIs and the newly approved third-generation inhibitor osimertinib. These models can be used to refine treatment strategies in patients with acquired resistance to first-line EGFR TKIs with primary or acquired resistance to osimertinib.

Serial droplet digital PCR (ddPCR) of plasma cell-free DNA (cfDNA) as pharmacodynamic biomarker in Phase 1 clinical trials for patients (pts) with *KRAS* mutant Non-Small Cell Lung Cancer (NSCLC)

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Introduction: Phase 1 clinical trials of novel therapeutics have historically focused on toxicity, but increasingly are doubling as efficacy studies in biomarker-enriched populations. Given the small sample sizes (often 3-6 patients per dose), response on imaging may be a coarse marker of therapeutic effect. Here we piloted serial ddPCR of plasma cfDNA as a pharmacodynamic (PD) marker in a phase I combination study of a MEK inhibitor and a CDK 4/6 inhibitor in patients with *RAS* mutated cancers.

Methods / Results: Twenty-five pts with *RAS*-mutated cancer (including 17 patients with *KRAS*-mutant non-small cell lung cancer) have been enrolled to date in a phase I dose escalation trial of the MEK inhibitor PD-0325901 with the CDK4/6 inhibitor palbociclib (NCT02022982). Plasma for cfDNA genotyping was collected at baseline prior to therapy and at the beginning of cycle 2. Plasma genotyping for *KRAS* G12X mutations was performed using a validated and highly quantitative droplet digital PCR assay.

Pts were enrolled in 5 dose level cohorts ranging from 75 mg palbociclib daily (3 weeks on, 1 week off) with 2 mg PD-0325901 BID (3 weeks on 1week off) to 125 mg palbociclib daily with 8 mg PD-0325901 BID (Table). *KRAS* mutations were detected in 14/24 pts at baseline (59%, median 1402 copies/mL plasma, range: 11-93000), consistent with the previously reported sensitivity of 64%. A second blood draw at cycle 2 was obtained for all 14 pts. A positive plasma response, defined as decrease of KRAS G12X mutants from first to second dose, was observed in 6 pts (range $^{-}$6% - $^{-}$100%) with the most plasma responders (n=4 pts) at the maximum administered dose. At lower administered doses, there was a median increase in plasma *KRAS* mutant levels.

Dose Level	Palbociclib (QD, 3 wks on 1 wk off) *mg*	PD-0325901 (BID, 3 wks on 1 wk off) *mg*	N Enrolled (N analyzed)	Median Plasma change KRAS mut(%)
1-1	75	2	3 (1)	+24
1-2	75	4	3 (3)	+60 (range $^{+}$31 - $^{+}$150)
1-3	100	4	8 (4)	+150 (range $^{-}$6 - $^{+}$341)
1-4	125	4	4 (2)	+9 (range $^{-}$45 - $^{+}$61)
1-5	125	8	7 (4)	-27 (range $^{-}$7 – $^{-}$43)

Conclusions: Increasing dose levels resulted in more consistent decreases in KRAS mutation in cfDNA, consistent with a dose-dependent pharmacodynamic effect.These results highlight the potential value of serial plasma ddPCR as a PD marker in early phase clinical trials.