Clinical potential of circulating tumor DNA (ctDNA)–based molecular response (MR) and baseline blood-based tumor mutational burden (bTMB) for monitoring response to first-line (1L) chemoimmunotherapy in advanced squamous non-small cell lung cancer (sqNSCLC)

F. Ciardiello,[1]* Z. Feng,[2]* N. R. Smith,[3] Z. Andric,[4] S. A. Shell,[5] A. Yablonovitch,[6] G. Guezel,[7] J. Scheuenpflug[3]

***Co-first authors**

[1]Division of Medical Oncology, The University of Campania Luigi Vanvitelli, Napoli, Italy; [2]Global Clinical Biomarkers and Companion Diagnostics, EMD Serono Research & Development Institute, Inc., Billerica, MA, USA, an affiliate of Merck KGaA; [3]Global Clinical Biomarkers and Companion Diagnostics, Merck Healthcare KGaA, Darmstadt, Germany; [4]Medical Oncology, Clinical Hospital Center Bezanijska Kosa, Belgrade, Serbia; [5]Medical Affairs, Guardant Health, Redwood City, CA, USA; [6]Bioinformatics, Guardant Health, Redwood City, CA, USA; [7]Global Clinical Development, Merck Healthcare KGaA, Darmstadt, Germany

SCOPE

- **To monitor and predict response during immunotherapy-related combination therapy and evaluate the clinical utility of ctDNA-based approaches, such as bTMB and MR, using longitudinal cohort samples from a phase 2a study of patients with treatment-naive advanced sqNSCLC treated with 1L avelumab and cetuximab in combination with platinum-based doublet chemotherapy (NCT03717155)**

CONCLUSIONS

- **Plasma bTMB-high (≥20 mutations per megabase [mut/Mb]) as a predictor of immunotherapy, in combination with putative predictive biomarkers, has the potential to identify patients with advanced sqNSCLC that could benefit from 1L avelumab and cetuximab in combination with cisplatin and gemcitabine**
- **Plasma ctDNA analysis supports MR assessment in patients treated with immunotherapy-related combination therapy, indicating its potential clinical utility as an adjunct to RECIST in monitoring tumor response**

Correspondence: **Fortunato Ciardiello, fortunato.ciardiello@unicampania.it**
Zheng Feng, zheng.feng@emdserono.com

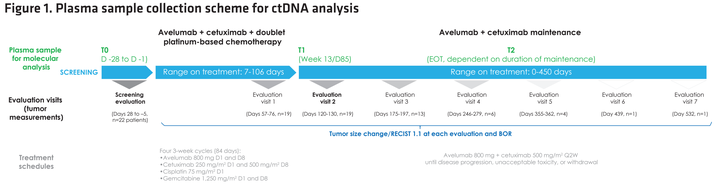
BACKGROUND

- The combination of avelumab, cetuximab, cisplatin, and gemcitabine showed clinical activity and a manageable safety profile with no additional safety signals for avelumab or cetuximab in a phase 2a study in 1L metastatic sqNSCLC[1]
- The availability of biomarkers for response to immunotherapy-related combination therapy could potentially predict survival benefit with 1L treatment for advanced sqNSCLC
- The patient-centric liquid biopsy (LBx) approach to detect changes in ctDNA could provide an early indication of treatment response to therapies and is an emerging tool to aid clinicians in treatment decision-making for various tumor types[2]

METHODS

- 52 longitudinal plasma samples (covering baseline, day 85 post treatment, and end of treatment) were collected from 22 consenting patients with advanced sqNSCLC who had been treated with 1L avelumab and cetuximab in combination with cisplatin and gemcitabine for four 3-week cycles (84 days) followed by a maintenance schedule using avelumab and cetuximab (0-451 days)[1] (**Figure 1**)
- Radiographic assessments per RECIST 1.1 were obtained at each evaluation visit for patients who remained on therapy. Confirmed best overall response (BOR) per RECIST 1.1 was available for 18 patients
- Circulating free DNA (cfDNA) was extracted and tested using the GuardantOMNI[3,4] (2.145 Mb) LBx panel to detect somatic alterations in 497 genes and generate bTMB from baseline samples and MR scores from baseline and day 85 samples
- bTMB-high was defined as ≥20 mut/Mb, and MR scores were calculated using a modification to the validated Guardant360 Response algorithm[5] by including qualifying somatic alterations across the GuardantOMNI panel. Patients were evaluable for MR by having somatic alterations at each time point that exceeded the threshold required for reliable MR score calculation

Figure 1. Plasma sample collection scheme for ctDNA analysis



BOR, best overall response; **ctDNA**, circulating tumor DNA; **D**, day; **EOT**, end of therapy; **Q2W**, every 2 weeks; **T**, sample collection time point.

RESULTS

bTMB and predictive biomarkers of immunotherapy response (**Figure 2**)

- 5 of 18 baseline samples were defined as bTMB-high, and all bTMB-high patients had a BOR of partial response (PR; n=3) or stable disease (SD; n=2). No bTMB-high patients had a BOR of progressive disease (PD)
- 13 of 18 baseline samples were bTMB-low, of which 10 had a BOR of PR (n=2) or SD (n=8) and 3 had a BOR of PD

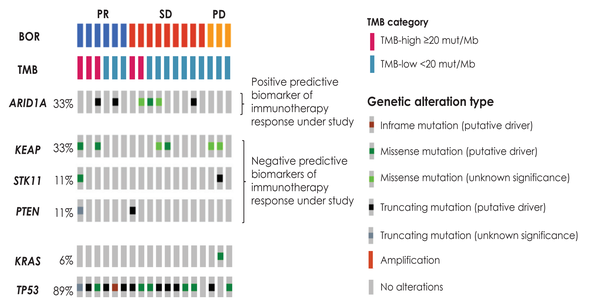
Genetic predictive biomarkers of immunotherapy response

- **Positive predictive biomarkers of response:** Mutation (*ARID1A*) was present in 7 of 18 baseline samples, all of which had a BOR of PR or SD. *ARID1A* mutations were present in 4 of 13 bTMB-low and 2 of 5 bTMB-high cases
- **Negative predictive biomarkers of response:** Mutations (*STK11, KEAP1, PTEN*) were present in 7 of 18 baseline samples, 5 of which had a BOR of PR or SD and 2 had non-PD
- Although the sample number is small, the presence or absence of positive response mutations was deemed potentially more informative in defining the response to immunotherapy-related combination therapy than negative response mutations

bTMB combined with positive predictive biomarkers of immunotherapy response

- Biomarker-positive patients were defined as those with bTMB-high and/or mutations in the positive predictive biomarkers of immunotherapy response, *ARID1A* mutations
  - 9 of 18 baseline samples were biomarker positive; all biomarker-positive patients had a BOR of PR or SD, and none had PD
  - The other 9 baseline samples were biomarker negative; 6 had a BOR of PR or SD, and 3 had a BOR of PD
- All 3 patients who had PD were bTMB-low, and 1 patient had a dual *STK11*/*KRAS* alteration, which was previously shown to negatively affect the clinical benefit of chemotherapy + immunotherapy[6]
- In this small data set, the presence or absence of mutations in positive predictive biomarkers combined with bTMB was deemed potentially more informative in defining the response to immunotherapy-related combination therapy than combining negative predictive biomarkers with bTMB

Figure 2. Predictive value of bTMB-high combined with predictive biomarkers of immunotherapy response

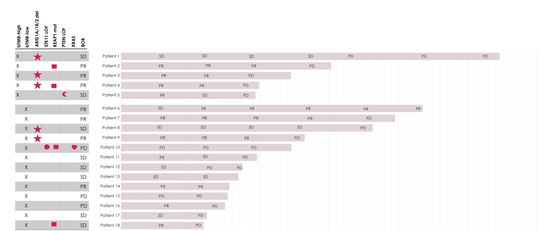


BOR, best overall response; **bTMB**, blood-based tumor mutational burden; **mut/Mb**, mutation per megabase; **PD**, progressive disease; **PR**, partial response; **SD**, stable disease; **TMB**, tumor mutational burden.

Time on treatment with chemoimmunotherapy (**Figure 3**)

- For the 5 of 18 patients with bTMB-high, average time on treatment was 297 days (SD ± 148)
  - 3 patients had a mutation in *ARID1A*, *ARID1B*, or *ARID2*, 2 had *KEAP1* mutations, and 1 had a *PTEN* loss of function (LOF) mutation
- For the 13 patients with bTMB-low, average time on treatment was 226 days (SD ± 107)
  - 1 patient, who had a BOR of PR, had *STK11* LOF, *KEAP1* mutation, and *KRAS* activation alterations, which have been associated with diminished efficacy of immunotherapy in lung adenocarcinoma[6]

Figure 3. Time on treatment

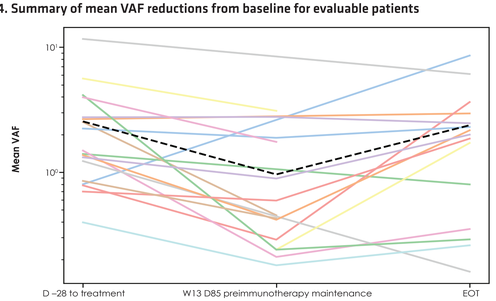


Not all patients had samples collected at each time point.
BOR, best overall response; **bTMB**, blood-based tumor mutational burden; **PD**, progressive disease; **PR**, partial response; **SD**, stable disease.

Longitudinal change of mean variant allele frequency (VAF)

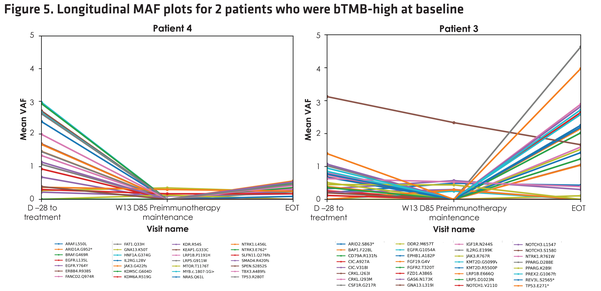
- 21 of 22 patients were evaluable for MR by having somatic alterations at each time point that exceeded the threshold required for reliable MR score calculation (**Figure 4**)
- 1 patient with PR demonstrated mean VAF reduction from baseline to day 85 and EOT (**Figure 5**, **left panel**)
- 1 patient with PR demonstrated mean VAF reduction from baseline to day 85. Mean VAF increased at EOT (**Figure 5**, **right panel**)

Figure 4. Summary of mean VAF reductions from baseline for evaluable patients



Dashed line represents the mean value of all mean VAFs measured at that particular time point across all patients.
D, day; **EOT**, end of treatment; **VAF**, variant allele frequency; **W**, week.

Figure 5. Longitudinal MAF plots for 2 patients who were bTMB-high at baseline

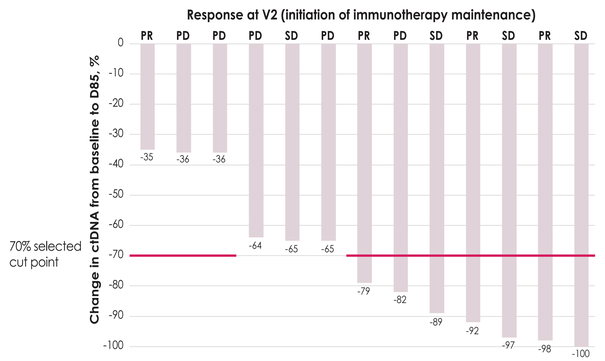


*Contains a nonsense mutation that results in premature termination of the protein sequence.
bTMB, blood-based tumor mutational burden; **D**, day; **EOT**, end of treatment; **MAF**, mutation annotation format; **VAF**, variant allele frequency; **W**, week.

Correlation of change in ctDNA percentage with response at initiation of maintenance

- 6 of 7 (86%) patients with ctDNA change at a selected cutoff ≥70% had a visit 2 (V2) response of PR (n=3) or SD (n=3)
- At a selected cutoff <70% for ctDNA change, 4 of 6 (67%) patients had a V2 response of PD
- The average ctDNA reduction from baseline to day 85 was significantly greater for patients classified as PR or SD than for patients with PD (82% vs 57%; p=0.032)

Figure 6. ctDNA percentage change from baseline and response at initiation of immunotherapy maintenance



ctDNA, circulating tumor DNA; **D**, day; **PD**, progressive disease; **PR**, partial response; **SD**, stable disease; **V2**, visit 2.

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