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A bug in the resistance to EGFR inhibitors: is there a role for Mycoplasma and cytidine deaminase in reducing the activity of osimertinib in lung cancer patients?

Alessandro Leonetti,[1,2,*] Daniela Carbone,[2,3,*] Alessandro Gregori,[2] Marcello Tiseo,[1] Godefridus J. Peters,[2,4] Dongmei Deng,[5] Elisa Giovannetti[2,6]

[1]Medical Oncology Unit, University Hospital of Parma, Parma, Italy; [2]Department of Medical Oncology, Amsterdam UMC, VU University, Cancer Center Amsterdam, Amsterdam, Netherlands; [3]Dipartimento di Scienze e Tecnologie Biologiche Chimiche e Farmaceutiche (STEBICEF), Università degli Studi di Palermo, Palermo, Italy; [4]Department of Biochemistry, Medical University of Gdansk, 80-211 Gdansk, Poland; [5]Department of Preventive Dentistry, Academic Centre for Dentistry Amsterdam (ACTA), University of Amsterdam and VU University Amsterdam, Amsterdam, The Netherlands; [6]Cancer Pharmacology Lab, AIRC Start-Up Unit, Fondazione Pisa per la Scienza Pisa, Pisa, Italy

Corresponding author

Elisa Giovannetti, MD, PhD

Department of Medical Oncology, Laboratory Medical Oncology, Cancer Center Amsterdam, Amsterdam UMC, Location VUmc, PO Box 7057, 1007 MB, Amsterdam, the Netherlands

E-mail address: e.giovannetti@amsterdamumc.nl

Dear Editor,

with great interest we read the article "*Mycoplasma hyorhinis* infection promotes tyrosine kinase inhibitor (TKI) resistance in lung adenocarcinoma patients", by Dai et al. [1].

This article describes a retrospective study evaluating the relationship between *Mycoplasma hyorhinis* infection and epidermal growth factor receptor (EGFR) TKI resistance in 101 patients with *EGFR*-mutated lung adenocarcinoma. *Mycoplasma hyorhinis* infection was detected by immunohistochemistry using the monoclonal antibody PD4, which specifically recognizes a distinct protein of *Mycoplasma hyorhinis*. The results showed that 60% of the tissues were positive for *Mycoplasma hyorhinis*, and this group of patients had a significantly shorter median progression-free survival (PFS) compared to the *Mycoplasma hyorhinis* negative cases.

This data yields relevant clinical perspectives, including new potential therapeutic strategies to decrease *Mycoplasma hyorhinis* infection in order to reduce EGFR-TKI resistance. However, in addition to appreciating the authors' efforts, we also discuss some key points, which will hopefully contribute to the validity of future studies within this field of research.

Firstly, the study population is largely heterogeneous, including both treatment-naïve and previously treated patients, across different treatment strategies. This information could help to understand the influence of treatment on lung microbiome, as suggested by previous studies [2]. However, the authors did not provide information concerning the site of specimen collection (i.e primary tumor, lymph node, metastatic lesion). In addition, one part of specimens consisted of cytology samples, and we wonder whether the researchers could validate the cytology smears in tissue immunohistochemistry, in at least a subgroup of their cases. This will indeed pave the way for future studies with objective and reproducible criteria for the standardized assessment of *Mycoplasma* infection in different specimens.

Other important questions arise regarding the clinicopathological features of the patients. A significant difference in smoking status was observed between *Mycoplasma*-positive and *Mycoplasma*-negative groups, with the *Mycoplasma hyorhinis* infection ratio being significantly higher in smokers than non-smokers ($p<0.001$), and we assume that this imbalance could also have influenced the results. Indeed, non-smoking status has been

associated with longer PFS than ever smoking after EGFR-TKIs treatment [3]. In addition, a total of 11 patients with *EGFR* exon 20 insertions were included in this analysis, while *EGFR* ex20ins is most resistant to EGFR-TKI [4]. While none of the patients in the *Mycoplasma*-negative group experience progression of disease at the first and third month following TKI, we can not exclude that poor responses observed in *Mycoplasma*-positive group were influenced by this peculiar type of EGFR mutation. Therefore, prospective studies in larger cohorts of patients without imbalance of relevant clinical and genetic characteristics are warranted.

However, we have a major concern about the hypothesis that pyrimidine-based EGFR-TKIs, such as osimertinib, can be hydrolyzed through the *Mycoplasma* enzyme cytidine deaminase (CDA) because several TKIs contain a structure similar to the cytosine ring of a pyrimidine as found in gemcitabine, leading to drug resistance and disease progression. The role of microbial CDA in resistance to gemcitabine was described earlier in pancreatic and colorectal cancer models [5,6]. However, osimertinib cannot be a substrate for the deamination reaction since the TKI lacks both the sugar moiety and the free amino group, as shown in Figure 1.

In conclusion, we are indebted to Dai and collaborators for their research on the implications of *Mycoplasma* infection on disease recurrence in lung cancer patients treated with EGFR-TKIs. This observational study provides a strong rationale for future trials, but standardized techniques of sample collection and analysis, larger populations, according to powered statistical analysis, and integration with functional data are essential to strengthen the value of *Mycoplasma* in clinics beyond already available mechanisms that underly EGFR-TKI resistance [7]. These studies will hopefully translate into improved therapies and clinical outcome in *EGFR*-mutated lung cancer patients.

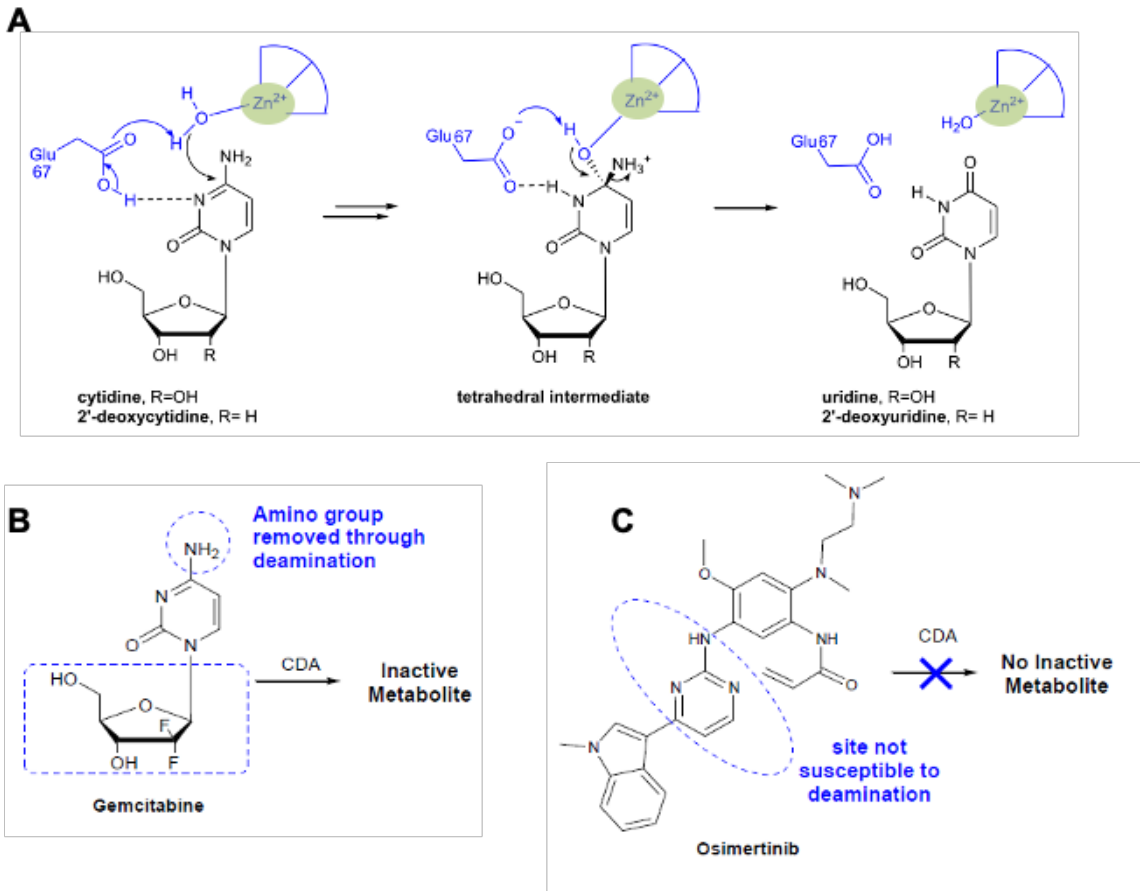

Figure 1. (A) The active-site hydrogen-bonding interactions between cytidine and CDA and the transition-state for deamination of cytidine to uridine. The Zinc-dependent cytidine deaminase (CDA) catalyzes the conversion of the free amino group of cytidine into a carbonyl moiety, forming the nucleoside uridine, through a two-steps sequential reaction. The first step involves the proton transfer from the zinc-coordinated water to Glu67, following by rotation of the carboxyl group of Glu67 and second proton transfer from the Glu67 to cytidine. A subsequent nucleophilic attack by the zinc-bound hydroxyl group at the C4 of cytidine generates a tetrahedral intermediate. The second step concerns the proton transfers from the zinc-bound hydroxide anion to Glu67 and from Glu67 to the amino group, with the release of ammonia and the concomitant departure of uridine from the active site of CDA. **(B) CDA-mediated inactivation of gemcitabine.** Gemcitabine is metabolically inactivated by CDA, limiting its therapeutic use [8]. Mechanistic studies revealed that, besides the free amino group on the cytosine base moiety, also the sugar moiety plays an important role, enhancing the zinc-promoted hydration due to the high electronic nature of the aglycone, which is effectively transmitted to the sugar moiety through the anomeric effect and orbital interactions between the oxygen's ion pair of pentofuranose moiety (O4') with the highest p orbital component and the adjacent beta-N1-glycosidic bond [9]. As a result, the absence of the O4'oxygen abolishes the stereoelectronic cooperation between the cytosine and the ribofuranosyl moiety, reducing the propensity of the base to form a covalent hydrate tetrahedral intermediate. Consequently, replacement of the sugar ring (ribose or 2'-deoxyribose) with carbocyclic pseudosugars removes any interplay between the sugar and the nucleobase, resulting into decrease or lack in deamination rates [10]. **(C) CDA cannot mediate the inactivation of osimertinib.** Osimertinib is not a suitable substrate for the deamination reaction by CDA because it lacks both the sugar moiety and the free amino group.

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