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Original Research

SARS-CoV-2 infection in cancer patients on active therapy after the booster dose of mRNA vaccines

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Abstract ***Introduction:*** The protective role against SARS-CoV-2 infection by the third booster dose of mRNA vaccines in cancer patients with solid malignancies is presently unknown. We prospectively investigated the occurrence of COVID-19 in cancer patients on active therapy after the booster vaccine dose.

Methods: Cancer patients on treatment at the Center for Immuno-Oncology (CIO) of the University Hospital of Siena, Italy, and health care workers at CIO who had received a booster third dose of mRNA vaccine entered a systematic follow-up monitoring period to prospectively assess their potential risk of SARS-CoV-2 infection. Serological and microneutralization assay were utilized to assess levels of anti-spike IgG, and of neutralizing antibodies to the SARS-CoV-2 Wild Type, Delta and Omicron variants, respectively, after the booster dose and after negativization of the nasopharyngeal swab for those who had developed COVID-19.

Results: Ninety cancer patients with solid tumors on active treatment (Cohort 1) and 30 health care workers (Cohort 2) underwent a booster third dose of mRNA vaccine. After the booster dose, the median value of anti-spike IgG was higher ($p = 0.009$) in patients than in healthy subjects. Remarkably, 11/90 (12%) patients and 11/30 (37%) healthy subjects tested positive to SARS-CoV-2 infection during the monitoring period. Similar levels of anti-spike

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IgG and of neutralizing antibodies against all the investigated variants, with geometric mean titers of neutralizing antibodies against the Omicron being the lowest were detected after the booster dose and after COVID-19 in both Cohorts.
Conclusions: The occurrence of SARS-CoV-2 infection we observed in a sizable proportion of booster-dosed cancer patients and in healthy subjects during the Omicron outbreak indicates that highly specific vaccines against SARS-CoV-2 variants are urgently required.

1. Introduction

Since the very beginning of the SARS-Cov-2 pandemic, oncologists have paid great attention to the potential interference of COVID-19 on the clinical course of the disease [1] and ongoing therapies in cancer patients [2]. Major concerns were raised by immune-checkpoint (ICI) therapy due to its ability to boost patients' immune response [3], and the potential radiologic overlaps between ICI- and COVID-19-induced pneumonitis [4]. In the absence of specific guidelines, the initial reports of patients who successfully resumed ICI therapy after COVID-19 were encouraging [5]. In that uncertain scenario, the swift availability of mRNA vaccines to SARS-COV-2 raised the additional question about their immunologic efficacy in cancer patients, particularly in those on active anti-neoplastic therapy. Thus, vaccination campaigns for cancer patients were complemented by a number of studies demonstrating the ability of mRNA vaccines to progressively induce and boost the titres of circulating anti-spike IgG and of neutralising antibodies, along with subsequent mRNA vaccine doses [6,7]; nevertheless, seroconversion seemed to be impaired by patients' performance status [8]. On our hand, investigating a longitudinally-followed group of 131 cancer patients with solid tumours on active therapy, we found similar anti-spike IgG titres in patients and in a control group of 42 vaccinated health care workers after the second dose of mRNA vaccine; of note, median values of anti-spike IgG were significantly higher in patients undergoing immune checkpoint(s) (ICI) therapy, as compared to those receiving chemotherapy or targeted therapy plus ICI [9].

The mandated booster vaccination with mRNA vaccines mRNA-1273 (Moderna) and BNT162b2 (Pfizer) was shown to significantly increase the titre of neutralising antibodies to SARS-CoV-2 wild type (WT) and Delta variants and to a lesser extent to the recently emerged Omicron variant, both in cancer patients on active therapy [10] and in healthy subjects, compared to the second dose [11,12]. A protective role of a third mRNA vaccine dose against SARS-CoV-2 infection was initially reported in healthy volunteers before the outbreak of the Omicron variant [13]; however, a further vaccination showed low efficacy in preventing SARS-CoV-2 infection during the outburst of the Omicron variant [14]. In this scenario, the protective role against SARS-CoV-2 infection of the third booster vaccination in cancer patients on active therapy is highly relevant and remains to be assessed [10], along with the debated suitability for a fourth dose with available mRNA vaccines in these highly fragile patients.

We now report the efficacy of the booster mRNA vaccine dose in preventing SARS-CoV-2 infection in cancer patients with solid malignancies on active therapy.

2. Methods

2.1. Participants

Cancer patients on active therapy at the Center for Immuno-Oncology (CIO) at the University Hospital of Siena, Italy, and health care workers at CIO underwent a booster third dose of mRNA vaccine. After this booster dose, all subjects entered a systematic follow-up monitoring period to prospectively assess their potential risk of SARS-CoV-2 infection. As per institutional guidelines, patients and healthy subjects tested negative for nasopharyngeal swabs within 48 h prior to hospital admissions and every 10 days, respectively. Sera were collected from blood drawings from routine workup after the booster dose and after the negativisation of the nasopharyngeal swab for those who had developed COVID-19. Clinical manifestations of COVID-19 were assessed according to the COVID-19 Treatment Guidelines. National Institutes of Health [15].

2.2. Serologic assays and statistical methods

Circulating levels of anti-spike IgG were determined using the Abbott SARS-CoV-2 IgG II Quant assay (Abbott Laboratories, Chicago, IL), a chemiluminescent microparticle immunoassay used as an aid in evaluating the immune status of individuals with quantitative measurement of IgG antibodies against the spike receptor-binding domain of SARS-CoV-2. This assay was performed on an Abbott Alinity (Abbott

Diagnostics) according to the manufacturer's instructions. A sample was considered positive when the result was >50.0 AU/ml. Values higher than 40,000 AU/ml were not further investigated and were reported as 40,000, being the upper limit of the kit detection. All data are represented as median with a two-sided 95% CI estimated using a normal approximation. Statistical significance for differences between cancer patients and healthy controls was carried out using a nonparametric two-sided Mann–Whitney test.

Titres of neutralising antibodies to SARS-CoV-2 WT, Delta and Omicron variants in cancer patients and healthy subjects were carried out by microneutralisation assay on Vero E6 cells in a 96-well microplate. Twenty-five microlitres of two-fold serial dilutions (1:8 to 1:2048) of sera samples were added to an equal volume of the SARS-CoV-2 strain WT (SARS-CoV-2/human/ITA/Siena-1/2020; GenBank: MT531537.2), Delta (SARS-CoV-2/human/ITA/TUS-Siena-40/2021; GenBank: OM736177.1), and Omicron (SARS-CoV-2/human/ITA/TUS-Siena5324294/2022; GenBank: OM956353) containing 100 $TCID_{50}$ and incubated for 90 min at 37 °C. Finally, 50 µl of Vero E6 cells suspension (2×10^5 cells/ml) prepared in complete DMEM were added to each well. After incubation at 37 °C, cultures were examined daily for the presence of cytopathic effect under a microscope (Olympus IX51). The 50% endpoint titre was calculated using the Reed–Muench method. A positive and a negative control serum were included in each assay. Geometric mean titres (GMTs) of the neutralisation assays were calculated. Nonparametric two-sided Mann–Whitney test was performed to detect significant differences in neutralising titre against WT, Delta and Omicron variants in cancer patients and healthy subjects.

The correlation between titres of neutralising antibodies to SARS-CoV-2 WT, Delta and Omicron variants and levels of circulating anti-spike IgG in cancer patients and healthy subjects was evaluated by Pearson's correlation coefficient (r). The p values <0.05 were considered statistically significant. Statistical analyses were carried out by GraphPad Prism 8 (GraphPad Software Inc., San Diego, CA).

3. Results

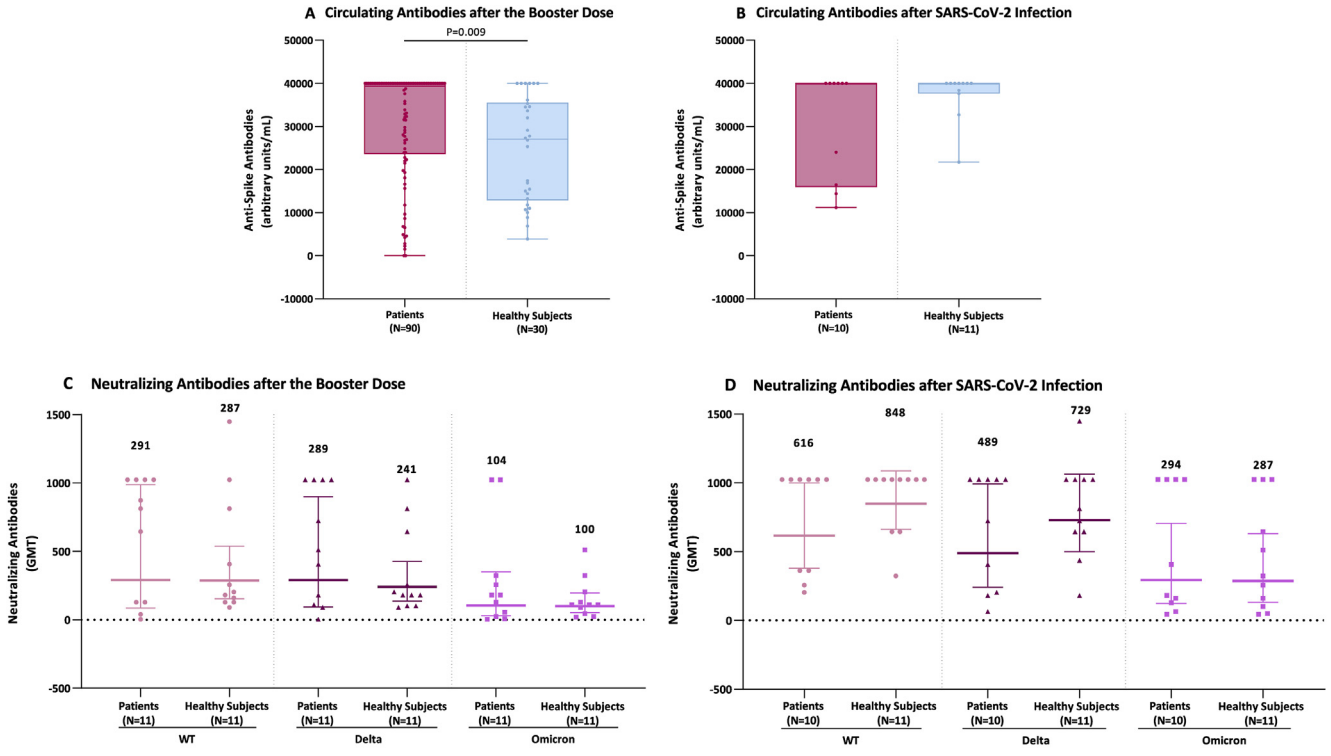
Ninety cancer patients (Cohort 1) on active treatment and 30 health care workers (Cohort 2) (Table S1) underwent a booster third dose of mRNA vaccine (Cohort 1, 98% mRNA-1273, 1% mRNA BNT162b2 and 1% heterologous; Cohort 2, 80% BNT162b2 and 20% heterologous). Being categorised as fragile or at high-risk subjects per Health Authorities' indication, a third vaccination was administered early on to cancer patients that were dosed between September 23 and December 21, 2021 (median 153 days after the second dose; range 138–235), and subsequently to health care workers between October 18 and December 6, 2021 (median 277 days after the second dose; range 241–307). After this booster dose, subjects from both Cohorts entered a systematic follow-up monitoring period to prospectively assess their risk of SARS-CoV-2 infection. Remarkably, from December 22, 2021, to January 27, 2022, 11/90 (12%) patients on therapy (Table 1) and 11/30 (37%) healthy subjects were tested positive to SARS-CoV-2 infection by a nasopharyngeal swab; conversely, only 1/131 (0.8%) patient and 1/42 (2.4%) control had developed SARS-CoV-2 infection after the second dose, respectively, in July and May, 2021; thus, ahead of the emergence of the Omicron variant.

The high rate of patients and healthy controls infected after the booster dose, and in a short period of time in which the SARS-CoV-2 Omicron variant was dominant prompted us to assess their levels of anti-spike IgG and of neutralising anti-SARS-CoV-2 antibodies against the WT virus, and its Delta and Omicron variants. To this end, sera were collected after the booster dose (Cohort 1, median 16 days, range 5–30; Cohort 2, median 41 days; range 14–62) and after the negativisation of the nasopharyngeal swab (Cohort 1, median 9 days, range 6–14; Cohort 2, median 7 days, range 7–9) were analysed. One patient with concurrent cutaneous melanoma and non-Hodgkin B lymphoma undergoing anti-CD20 therapy was removed from post-infection assessments, being still positive for SARS-CoV-2 infection at the time of analysis. After the booster dose, the median value of anti-spike IgG was higher (p = 0.009) in patients

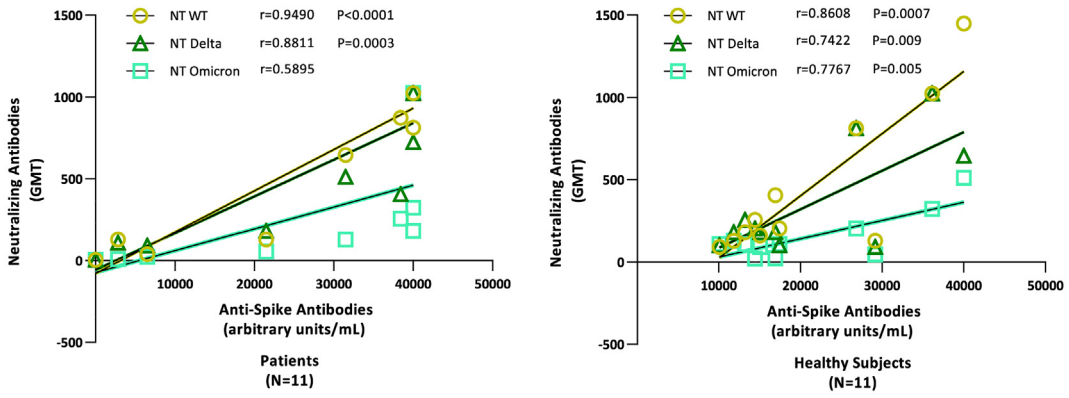
Table 1
Clinical features and COVID-19-related symptoms of cancer patients and healthy subjects after SARS-CoV-2 infection.

	Cohort 1 (N = 11)	Cohort 2 (N = 11)
Gender		
Male	64% (7)	27% (3)
Female	36% (4)	73% (8)
Median age, years (range)	58 (43–77)	33 (27–64)
Cancer histology		NA
Melanoma	64% (7)	
Breast cancer	9% (1)	
Glioblastoma Multiforme	9% (1)	
Endometrial Cancer	9% (1)	
Angiosarcoma	9% (1)	
Cancer treatment		NA
ICI	64% (7)	
TT	27% (3)	
CT	9% (1)	
Median time-to-negativisation of nasopharyngeal swabs, days (range)	14 (6–29)	10 (7–19)
COVID-19-related symptoms		
Asymptomatic	36% (4)	18% (2)
Mild	55% (6)	73% (8)
Moderate	9% (1)	9% (1)
Severe	0	0

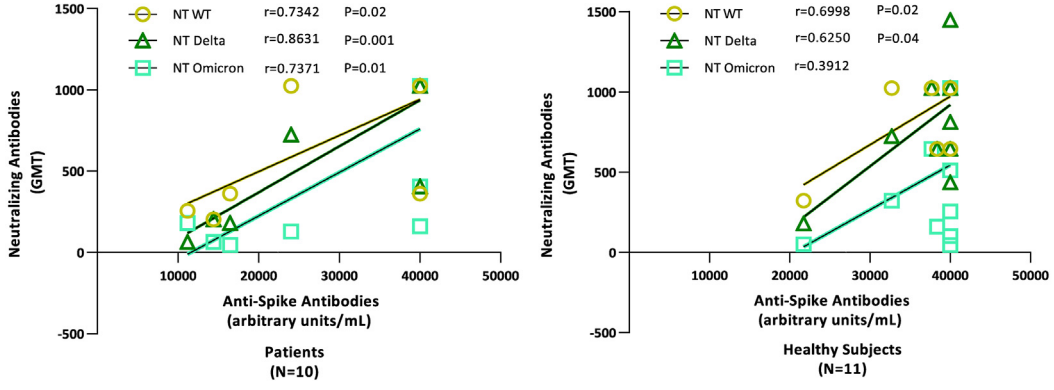
ICI = immune checkpoint(s) inhibitors; TT = targeted therapy; CT = chemotherapy.
NA = not applicable.



E Correlation between Anti-Spike and Neutralizing Antibodies in Patients and Healthy Subjects after the Booster Dose



F Correlation between Anti-Spike and Neutralizing Antibodies in Patients and Healthy Subjects after SARS-CoV-2 Infection



than in healthy subjects [Cohort 1, 39,388 arbitrary units per millilitre (AU/ml), 95% confidence interval (CI) 32,159–40,000; Cohort 2, 27,073 AU/ml, 95% CI 15,037-34,534] (Fig. 1A); SARS-CoV-2 infection increased the median value of anti-spike IgG to the same extent in both groups (Cohort 1, 40,000 AU/ml, 95% CI 14,412–40,000; Cohort 2, 40,000 AU/ml, 95% CI 32,724–40,000) (Fig. 1B).

Interestingly, SARS-CoV-2 infection increased the levels of neutralising antibodies against all the investigated variants compared to the booster dose in both Cohorts (Fig. 1C, D); importantly, however, the geometric mean titre of neutralising antibodies against all the investigated SARS-CoV-2 variants did not differ between cancer patients and healthy subjects, neither after SARS-CoV-2 infection nor after the third dose (Fig. 1C and D). Of relevance, the geometric mean titres of neutralising antibodies against the Omicron variant detected after SARS-CoV-2 infection remained the lowest in both Cohorts, as also observed after the third vaccine dose (Fig. 1C and D). Increasing titres of anti-spike IgG correlated with the increase of neutralising antibodies against all SARS-CoV-2 variants after the booster dose (Fig. 1E) and also after SARS-CoV-2 infection (Fig. 1F) in both Cohorts. Furthermore, in both the Cohorts, the median titres of anti-spike IgG after the booster dose did not significantly differ in SARS-CoV-2-infected individuals (Fig. S1A) from their median values in non-infected subjects (Fig. S1B).

The median time-to-negativisation of the nasopharyngeal swabs did not significantly differ between cancer patients and healthy subjects (Table 1), and COVID-19 related symptoms were mild in the majority of subjects in both Cohorts (Table 1), with no investigated subjects requiring hospitalisation or death due to COVID-19 (data not shown).

4. Discussion

The worldwide outburst of the highly infective SARS-CoV-2 Omicron variant in December 2021 [16] overlaps with the swift increase in the number of SARS-CoV-2-infected cancer patients and healthy controls that we observed in our two prospectively investigated Cohorts, despite of the booster dose. Remarkably, although limited by the sample size of our Cohorts, patients with solid tumours undergoing active therapy maintained an immunologically competent status to the booster dose of mRNA vaccine, like healthy subjects. In this context, the higher levels of anti-spike IgG we detected after the booster dose in patients as compared to healthy subjects could likely be due to the prevalence of ICI therapy [9], the shorter time-interval between booster dose and serum collection, and to the suggested higher immunological efficacy of the Moderna vaccine [17–19]. Further supporting the 'immunologic consistency' observed between patients and healthy subjects, SARS-CoV-2 infection-induced comparable levels of anti-spike IgG and of neutralising antibodies in both Cohorts. In this comprehensive scenario, the increasing titres of anti-spike IgG we observed in both Cohorts after the booster dose represent an interesting surrogate of an underlying increase of neutralising antibodies against all SARS-CoV-2 variants, although they unlikely seem to predict the occurrence of SARS-CoV-2 infection. Consistent with this hypothesis, increasing titres of anti-spike IgG positively correlated with neutralising antibodies against all analysed variants also after SARS-CoV-2 infection both in cancer patients and healthy subjects. Further support to this notion is provided by the median titres of anti-spike IgG in SARS-CoV-2-infected individuals that did not differ from their median values in non-infected subjects after the booster dose in both the Cohorts. Additionally, the median time-to-negativisation of the nasopharyngeal swabs did not significantly differ between Cohort 1 and Cohort 2, again suggesting that vaccine-boosted cancer patients were capable of efficiently mounting a protective immunity capable of clearing SARS-CoV-2 as healthy subjects did.

The occurrence of COVID-19 after the booster dose in a sizeable proportion of cancer patients on active therapy requires attention and needs to be further explored in large series investigated by international consortia. Nevertheless, the usefulness of a third vaccine dose in cancer patients seems to remain unquestionable at present [20–24], also due to the observed increase of neutralising antibodies to the Omicron variant [10–12], and to the generally mild clinical course of SARS-CoV-2

Fig. 1. Anti-spike IgG response and neutralising antibodies to SARS-CoV-2 WT, Delta and Omicron variants by a booster dose of mRNA vaccines and after SARS-CoV-2 infection in cancer patients and healthy subjects. Levels of circulating anti-spike IgG were assessed in cancer patients and in healthy subjects after the booster dose of the mRNA-1273 (Moderna) or BNT162b2 (Pfizer-BioNTech) vaccine and after SARS-CoV-2 infection. Differences between titres of anti-spike IgG in cancer patients and healthy subjects are reported (Panel A, B). In each box-and-whisker plot, the horizontal line represents the median, the top and bottom of the box show the interquartile range, and the whiskers show the minimum and maximum values. Each dot represents individual serum sample. Titres of neutralising antibodies to SARS-CoV-2 wild type (WT), Delta and Omicron variants in cancer patients and healthy subjects are reported in Panel C, D. In each scatter plot, GMTs (horizontal lines) with 95% CI are presented. Dots indicate individual serum samples. Correlation between titre of neutralising antibodies to SARS-CoV-2 WT, Delta and Omicron variants and levels of circulating anti-spike IgG was evaluated in cancer patients (Panel E, F) and healthy subjects (Panel E, F). Symbols indicate individual serum samples. The P values lt;0.05 were considered statistically significant.

infection that we observed, complying with the aim of vaccination to reduce COVID-19-related hospitalisations and deaths. Consistently, the booster dose was reported to be significantly associated with lower rates of symptomatic infections in healthy subjects compared to those who received only two vaccine doses [13].

In spite of the efficient immunologic response to SARS-CoV-2 after the third vaccination and infection and of the clinical course of COVID-19 we found in cancer patients with solid tumours on active therapy, the rate of SARS-CoV-2 infection we observed after the booster dose is somewhat far from what would be desirable. This latter notion is enforced by the high rate of SARS-CoV-2 infection recently reported in patients with solid and haematologic malignancies during the emergence of the Omicron variant [25].

Comprehensively our findings, although initial and limited by the sample size, together with the recently reported poor efficacy of a fourth vaccine dose in healthy subjects [14], raise concerns about the opportunity of further vaccination with available mRNA vaccines in these fragile subjects who already received a third booster dose. Consistent with this hypothesis, second-generation vaccination strategies are urgently demanded for cancer patients on active therapy who need special care due to their underlying disease.

5. Conclusions

In spite of the ongoing anti-cancer treatment, a booster dose of mRNA vaccines to SARS-CoV-2 increased the levels of circulating anti-spike IgG and of neutralising antibodies in cancer patients with solid tumours on active therapy, with titres similar to those of healthy subjects. However, the high rate of SARS-CoV-2 infection we observed both in patients and in healthy subjects during the outbreak of the Omicron variant mandates that more specific vaccination strategies against SARS-CoV-2 are urgently required.

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Authors contributions

AMDG, MM: conceptualisation, methodology, data analysis, interpretation, writing original draft, review and editing.

GG, GA, CG, VD, LC, MLF: data collection, data analysis, review and editing.

MGC: data analysis, interpretation, review and editing.

Conflict of interest statement

AMDG has served as a consultant and/or advisor to Incyte, Pierre Fabre, Glaxo Smith Kline, Bristol-Myers Squibb, Merck Sharp Dohme, and Sanofi and has received compensated educational activities from Bristol Myers Squibb, Merck Sharp Dohme, Pierre Fabre and Sanofi; LC served as an advisor to Bristol-Myers Squibb, and received compensated educational activities from Bristol-Myers Squibb and AstraZeneca; MM has served as a consultant and/or advisor to Roche, Bristol-Myers Squibb, Merck Sharp Dohme, Incyte, AstraZeneca, Amgen, Pierre Fabre, Eli Lilly, Glaxo Smith Kline, Sciclone, Sanofi, Alfasigma, and Merck Serono; and own shares in Epigen Therapeutics S.r.l.

GG, GA, CG, VD, MFL and MGC declare no competing interests.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejca.2022.05.018.

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