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ARTICLE

Biomarker and pharmacodynamic activity of the transforming growth factor-beta (TGFβ) inhibitor SAR439459 as monotherapy and in combination with cemiplimab in a phase I clinical study in patients with advanced solid tumors

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Abstract

SAR439459, a 'second-generation' human anti-transforming growth factor-beta (TGFβ) monoclonal antibody, inhibits all TGFβ isoforms and improves the antitumor activity of anti-programmed cell death protein-1 therapeutics. This study reports the pharmacodynamics (PD) and biomarker results from phase I/Ib first-in-human study of SAR439459 ± cemiplimab in patients with advanced solid tumors (NCT03192345). In dose-escalation phase (Part 1), SAR439459 was administered intravenously at increasing doses either every 2 weeks (Q2W) or every 3 weeks (Q3W) with cemiplimab IV at 3 mg/kg Q2W or 350 mg Q3W, respectively, in patients with advanced solid tumors. In dose-expansion phase (Part 2), patients with melanoma received SAR439459 IV Q3W at preliminary recommended phase II dose (pRP2D) of 22.5/7.5 mg/kg or at 22.5 mg/kg with cemiplimab 350 mg IV Q3W. Tumor biopsy and peripheral blood samples were collected for exploratory biomarker analyses to assess target engagement and PD, and results were correlated with patients' clinical parameters. SAR439459 ± cemiplimab showed decreased plasma and tissue TGFβ, downregulation of TGFβ-pathway activation signature, modulation of peripheral natural killer (NK) and T

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cell expansion, proliferation, and increased secretion of CXCL10. Conversion of tumor tissue samples from 'immune-excluded' to 'immune-infiltrated' phenotype in a representative patient with melanoma SAR439459 22.5 mg/kg with cemiplimab was observed. In paired tumor and plasma, active and total TGFβ1 was more consistently elevated followed by TGFβ2, whereas TGFβ3 was only measurable (lower limit of quantitation ≥2.68 pg/mg) in tumors. SAR439459 ± cemiplimab showed expected peripheral PD effects and TGFβ alteration. However, further studies are needed to identify biomarkers of response.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

Novel therapies that target the transforming growth factor-beta (TGFβ) and programmed cell death protein-1/programmed cell death ligand-1 (PD-1/PD-L1) pathway may offer a unique approach to engaging the immune system and hence have an effect on the pharmacodynamics (PD) and patient biomarker profile.

WHAT QUESTION DID THIS STUDY ADDRESS?

This study assessed the PD and biomarker results from phase I/Ib first-in-human study of SAR439459 monotherapy and in combination with cemiplimab in patients with advanced solid tumors (NCT03192345).

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

The study showed peripheral and tumoral PD effects as well as the cancer patient enrichment strategies from the phase I/Ib trial of SAR439459, as monotherapy and in combination with cemiplimab, in patients with advanced solid tumors.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

The current biomarker data contribute to our understanding of the lack of enough therapeutic benefit from the therapeutic treatment and the potential patient enrichment strategies to future clinical studies on TGFβ blockade therapy in cancer patients.

INTRODUCTION

Transforming growth factor-beta (TGFβ) is a key regulator of physiological processes and tumorigenesis by promoting tumor growth, remodeling, progression, and resistance to checkpoint blockade[1,2] through its role in the epithelial to mesenchymal transition (EMT).[3,4] TGFβ overexpression is correlated to poor overall survival in patients with anti-programmed cell death protein-1 (PD-1) therapy-resistant/refractory tumors.[5]

SAR439459 is a 'second-generation' human anti-TGFβ immunoglobulin G4 (IgG4) monoclonal antibody that inhibits all TGFβ isoforms.[5] In preclinical studies, SAR439459 in combination with anti-PD-1 therapy has been shown to improve the antitumor activity of anti-PD-1 therapeutics. Further, the co-administration of anti-TGFβ and anti-programmed cell death ligand (PD-L1) inhibitors in preclinical models has been shown to promote T cell infiltration, antitumor immunity, and increased survival.[6–8]

Cemiplimab, a human IgG4 antibody, subjugates the PD-1-pathway-mediated inhibition of the antitumor immune response by binding to the PD-1 expressed on T cells and blocking its interaction with programmed cell death ligand PD-L1 and PD-L2.[9]

The phase I/Ib first-in-human study (NCT03192345) evaluated the safety, pharmacokinetics (PK), pharmacodynamics (PD), and antitumor activity of SAR439459 alone and in combination with cemiplimab in patients with advanced solid tumors. Herein, we report the PD and biomarker results.

METHODS

Study design and patient population

This was the first-in-human, open-label, dose-escalation, dose-expansion study of SAR439459 as a single agent or

in combination with cemiplimab (NCT03192345). The study comprised two parts: dose escalation (Part 1) and dose expansion (Part 2) in selected advanced solid tumors (melanoma, non-small cell lung carcinoma [NSCLC], hepatocellular carcinoma [HCC], urothelial cancer [UC], and mesenchymal colorectal cancer [CRC]). In Parts 1A and 2A, SAR439459 as monotherapy was evaluated, whereas in Parts 1B and 2B, the combination of SAR439459 and cemiplimab was assessed. The screening period of 4 weeks was followed by the treatment period (Figure S1).

Patients with histologically confirmed, advanced unresectable, or metastatic solid tumors, who – in the opinion of the investigator – did not have a suitable alternative therapy, were included in the dose escalation (Part 1). Patients with advanced melanoma were included in the monotherapy dose-expansion part (Part 2A). For Part 2B, patients with select tumor types, including mesenchymal CRC, HCC, melanoma, NSCLC, and UC, were included (Table S1). All patients had to have a site amenable to biopsy and measurable disease per Response Evaluation Criteria in Solid Tumors (RECIST 1.1) criteria.

Study treatment

In Part 1A (dose escalation, monotherapy), SAR439459 was administered intravenously (i.v.) at increasing doses (0.05, 0.25, 1, 3, 10, and 15 mg/kg every 2 weeks [Q2W]). In Part 1B (dose escalation, combination), SAR439459 i.v. doses cleared from Part 1A (0.25, 1, 3, 10, 15 mg/kg Q2W and 22.5 mg/kg every 3 weeks [Q3W]) were explored in combination with cemiplimab IV Q2W at either 3 mg/kg Q2W or 350 mg Q3W using a 3 + 3 design in adult patients with advanced solid tumors.

In Part 2A (dose expansion, monotherapy), patients with advanced melanoma, who had failed prior anti-PD1 or anti-PD-L1 containing treatment, were randomized 1:1 to receive SAR439459 IV Q3W at the preliminary recommended phase II dose (pRP2D) of 22.5 mg/kg or the lower dose of 7.5 mg/kg in a 21-day cycle. Both dose levels were expected to provide preliminary population PK, trough concentrations, respectively, close to or above IC50 and IC90 for TGFβ inhibition, based on a preclinical PK/PD model.[10] In Part 2B (dose expansion, combination), patients with selected advanced solid tumors (post anti-PD-1 or anti-PD-L1 melanoma, NSCLC and HCC, anti-PD-L1-naïve UC, and mesenchymal CRC regardless of prior anti-PD-L1 treatment) received SAR439459 at pRP2D of 22.5 mg/kg and cemiplimab 350 mg i.v. Q3W.

Biomarkers

Tumor biopsy and peripheral blood samples were collected during the study for exploratory biomarker analyses to assess target engagement, as well as immune modulatory PD effects of SAR439459, and the results were correlated with patients' clinical parameters.

Tumor biopsy for biomarker analysis

Fresh tumor biopsies were collected at the site by either an excisional or core needle biopsy as determined by local site practices. Additionally, material from a fine needle aspiration was also accepted for this sample type. Fresh biopsies were collected during the screening period (ideally within 7 days before the first dose) and on Cycle 2 Day 8 (C2D8) (±3 days). These biopsies were optional in Part 1A and Part 1B but mandatory in Part 2A and Part 2B in all patients (unless clinically unfeasible after discussion with Sanofi's medical monitor).

In the absence of fresh biopsies, archival frozen tumor biopsies and/or archival formalin-fixed, paraffin-embedded (FFPE) tumor blocks (collected within the past 3 months without any intervening therapies) were accepted during the screening period. These frozen biopsy samples were then used for a specific biomarker analysis, such as target engagement and genomic profiling. An archival FFPE biopsy block was only used for immunohistochemistry.

Whole blood phenotyping

For whole blood immunophenotyping flow cytometric analysis, we used a T cell proliferation and activation as well as the TBNK true count panels. T cell panel includes key proliferation- and activation-related markers, such as CD45, CD3, CD4, CD8, human leukocyte antigen-DR isotype (HLA-DR), Ki67, that can detect activated CD8 T cells (HLA-DR+ $CD8^+$) and proliferating CD8 T cells (Ki67+ $CD8^+$). The TBNK panel can measure absolute cell count of CD3+, CD4+, $CD8^+$ T cells, CD19+ B cells, and CD16+ CD56+ NK cells. Patient blood samples were collected in Cytochex BCT tubes. After red blood cell lysis, cells were stained using fluorescently labeled antibodies specific for the surface markers listed above. Samples were subsequently fixed, permeabilized, and then stained with an anti-Ki67 antibody. Stained samples were analyzed and resulting data were then reported by the testing laboratory (Labcorp, Indianapolis, IN, USA).

Circulating immune cell composition

Deoxyribonucleic acid (DNA) was isolated from blood leukocytes to evaluate changes in immune cell subsets ($CD8^+$ T cells and CD56+ natural killer [NK] cells) in response to treatment by methylation-specific quantitative polymerase chain reaction analysis to identify specific cell types (Precision for Medicine [Epiontis], Berlin, Germany).[11]

Cytokine/chemokine assay

Interferon gamma-induced protein 10 (IP-10) was measured in human plasma samples (R&D System kits DIP100 and D6050) by Covance Translational Biomarker Solutions Laboratory (Greenfield, IN, USA).

Quantification of total and active TGFβ levels

Total and active TGFβ1, TGFβ2, and TGFβ3 were measured in plasma (collected in CTAD tubes) and tumor biopsies (flash frozen) using the TGFβ Premixed Magnetic Luminex Performance Assay (R&D Systems) and the Bio-Plex 200 suspension array system (Bio-Rad Laboratories).

Immunohistochemistry

Tumor tissues were analyzed to access changes in infiltrating immune cells (e.g., $CD8^+$ T cells and Tregs cells) and immune markers (e.g., PD-1 and PD-L1) in tumor FFPE specimens that were stained for CD8 (C8/144B, DakoM7103) using the Ventana BenchMark ULTRA autostainer and imaged on an Aperio ScanScope AT by NeoGenomics Laboratories (Aliso Viejo, CA, USA). The calculation of each cell type per biopsy region of interest (ROI) was reported as the total positive counts for that marker and normalized density for each ROI. Slide level density was calculated as the sum of marker-positive counts divided by the sum of quality check passed areas of all ROIs.

Transcriptomic analysis

Ribonucleic acid (RNA) was isolated from peripheral blood mononuclear cells or whole blood for transcriptomic analysis to assess TGFβ pathway signaling modulation by the treatment. TGFβ pathway activation was assessed with gene set variation analysis using an established and validated 159-gene expression signature of TGFβ pathway activation.[12]

Development of a consensus molecular subtyping classifier signature for CRC

A consensus molecular subtype 4 (CMS4) gene classifier was developed to identify mesenchymal CRC tumors. RNA-sequencing (RNA-seq) was used to profile a training set of 155 FFPE CRC samples from a commercial source. The same samples were profiled using the nCounter platform (NanoString, Seattle, WA, USA) to evaluate concordance with RNA-seq expression. The assay was validated at LabCorp's Center for Molecular Biology and Pathology in their Clinical Laboratory Improvement Amendment (CLIA)-certified laboratory (Research Triangle Park, NC, USA) and was used to prospectively screen archival FFPE tumor samples from patients with CRC for the CMS4 phenotype. Samples were then resequenced (RNA-seq) and analyzed with a set of 29 genes representative of tissue that has undergone an EMT. Archival FFPE specimens that were submitted during the study screening phase were available for 143 patients. Of these, 58 were identified as having the CMS4 phenotype, and 28 were resequenced and analyzed for concordance between the two-gene panel (smooth muscle actin alpha-2 and vimentin) and the larger gene signature.

Ethical statement

The study was conducted in compliance with the ethical principles founded in the international ethics guidelines, including the Declaration of Helsinki and the International Council for Harmonisation (ICH) of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines for Good Clinical Practice (GCP), as well as all applicable laws, rules, and regulations. Institutional Review Board (IRB) approved the protocol, and participant informed consents were approved by the IRB prior to the initiation of the study. Written informed consent was obtained from all study participants prior to enrollment.

Statistical analysis

Boxplots with datapoints and lines were used to visualize the distribution of PD biomarker at each timepoint (baseline and C2D8) and to reveal the change in trend after treatment. Box center and upper/lower lines indicate the median and upper/lower quartile, respectively. Vertical

lines above and below the box indicate 1.5 times the interquartile range.

A violin plot was used to visualize the distribution of active tumor and plasma TGFβ; expression level was computed by quantiles.

Cohen's kappa was performed to measure the concordance between tumor active TGFβ1 and plasma total TGFβ1 expression level stratified by median, with the number and percentage of patients in each category illustrated by a Sankey plot. Plots were generated using R software version 4.0.4. (R: A language and environment for statistical computing).

RESULTS

TGFβ modulation

SAR439459, alone and in combination with cemiplimab, showed decrease of plasma TGFβ1 levels by ≥90% at all dose levels (Figure 1a).[10] In addition, the concomitant inhibition of TGFβ1 was also observed in paired biopsy specimens from patients in Part 2 at 7.5 or 22.5 mg/kg dose levels (Figure 1b).

In addition, analysis of RNA-seq data from paired tumor biopsies revealed concomitant downregulation of the TGFβ pathway activation signature (Figure 2). This is consistent with the observed modulation of the TGFβ level in plasma and tumor samples, which confirms target engagement by SAR439459.

Modulation of $CD8^+$ T and NK cells, and proinflammatory chemokine IP-10

The immunophenotyping to overall T and NK cells revealed that SAR439459, alone and in combination with cemiplimab, induced modulation of peripheral NK- and T cell expansion and proliferation (Figure S2A) and expansion (Figure S3A). Concomitantly, enhanced plasma levels of proinflammatory cytokine and chemokines, such as CXCL-10, were observed in patients after treatment with SAR439459 alone and in combination with cemiplimab (Figures S2B, S3B). Nevertheless, the modulation of these biomarkers is not statistically significant.

Intratumoral $CD8^+$ T cells

Treatment with SAR439459 alone and in combination with cemiplimab showed that the median percentage of $CD8^+$ T cells in the tumor microenvironment was numerically greater than that at baseline; however, the response among individual tumors varied (Figure S4A). Nevertheless, it was also observed that SAR439459 22.5 mg/kg in combination with cemiplimab resulted in the conversion of tumor tissue samples from an 'immune-excluded' to an 'immune-infiltrated' phenotype in a representative patient with melanoma (Figure 3). Due to the limited sample size and lack of cemiplimab monotherapy (control arm), we cannot conclude the contribution of component from SAR439459 versus cemiplimab. In addition to CD8, we have also tested modulation of PD-L1 and forkhead box P3 (Foxp3) in the tumor microenvironment. However, we did not observe a clear trend of modulation of these markers, and we think this could be due to the limited sample and heterogeneous patient populations from the early phase I study (Figure S4B, S4C).

Patient selection based on CMS4 classifier for CRC cohort

In Part 1, SAR439459 alone and in combination with cemiplimab induced modulation of general NK and T cells in the blood. The two-gene classifier showed good concordance between RNA-seq and NanoString results (Figure 4a) with validated test specificity (Figure 4b).

Upon retrospective verification of the initial results, of 28 archival samples that were selected as having a CMS4 phenotype using the two-gene classifier, only three were re-classified as "other than CMS4" (one as CMS1 and two as CMS2) with the analysis of 29 genes of the full EMT gene set, corresponding to a false-positive rate of 10.7% (Figure 4c and Table S2).

Distribution of TGFβ levels in tumor and plasma, as well as their correlation

For this study, highly specific assays were developed and used to measure active TGFβ1, TGFβ2, and TGFβ3 in flash-frozen NSCLC tumors along with total TGFβ1 and TGFβ2 in paired plasma samples from each respective patient. Active and total TGFβ1 were more consistently found to be elevated in these samples followed by TGFβ2, whereas TGFβ3 was only measurable (lower limit of quantitation [LLOQ] ≥2.68 pg/mg) in tumors. Distribution and prevalence analysis of TGFβ1 in tumor and plasma identified various cutoff values, which may provide insight for patient stratification and selection threshold identification (Figure 5a,b). Efforts were made to correlate intratumoral TGFβ levels to those found in the periphery (plasma) with the goal to assess the prognostic value of plasma TGFβ levels as a potential surrogate to intratumoral expression in the prognosis (Figure 5c).

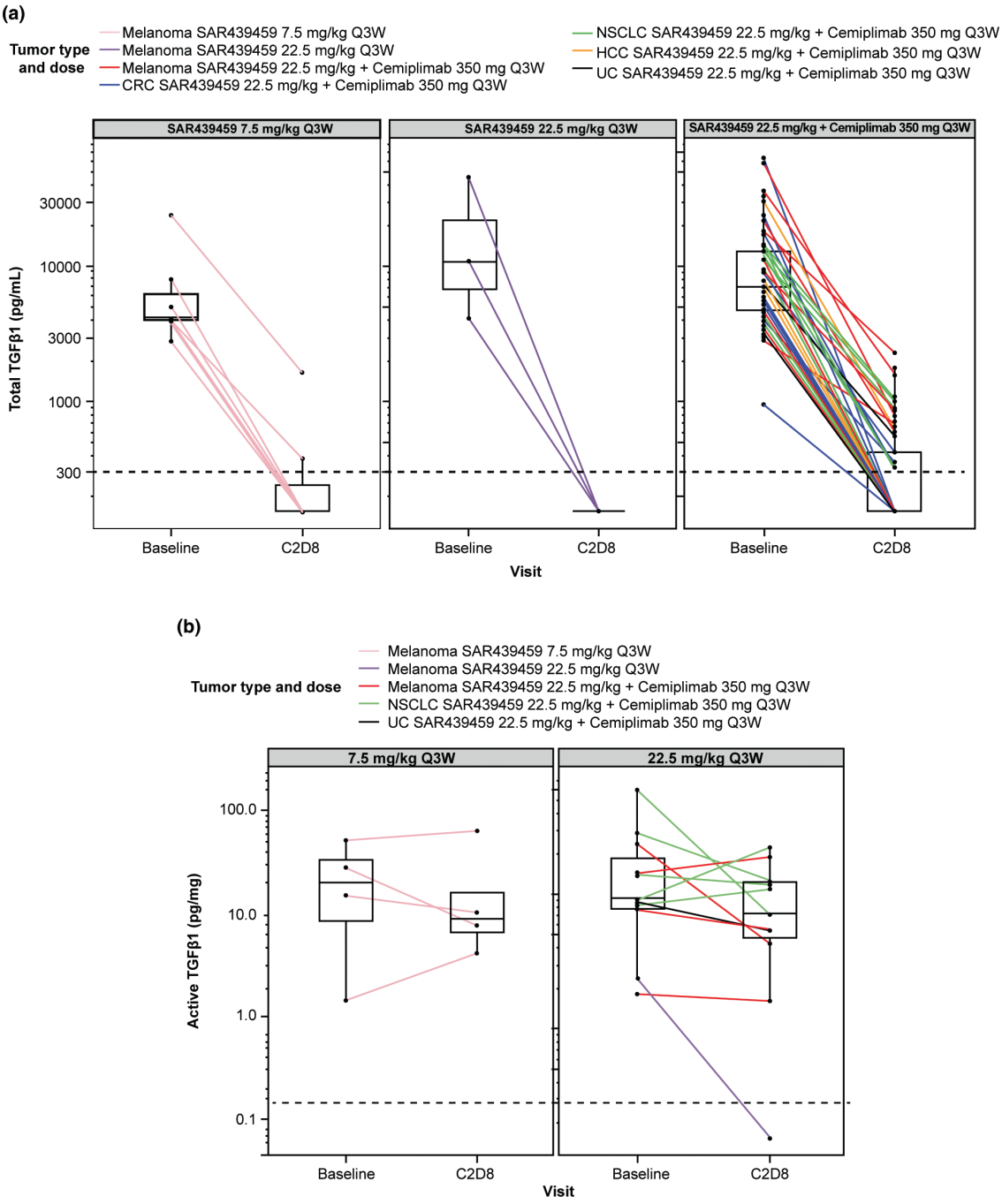

FIGURE 1 Modulation of (a) total transforming growth factor-beta (TGFβ1) in plasma and (b) active TGFβ1 in tumor tissue. Representative box plots showing the TGFβ modulation in Part 2:2 (a) SAR439459 7.5 mg/kg Q3W: $N = 7$; 22.5 mg/kg Q3W: $N = 3$; and SAR439459 22.5 mg/kg + cemiplimab 350 mg Q3W: $N = 53$; (b) SAR439459 7.5 mg/kg Q3W: $N = 4$ and 22.5 mg/kg Q3W: $N = 11$. Colored lines indicate individual patient plasma or tissue samples. The dashed line in each plot represents the lower limit of quantitation which is why all the points that are below these values (total and active) converge at one point as they are reported as below quantitative limit. C2D8, Cycle 2 Day 8; CRC, colorectal cancer; HCC, hepatocellular carcinoma; NSCLC, non-small cell lung carcinoma; Q3W, every 3 weeks; TGFβ, transforming growth factor-beta; UC, urothelial cancer.

Tumor-promoting activities of TGFβ within the tumor microenvironment, which include EMT, fibrosis, angiogenesis, and immunosuppression, are nonredundant with the tumor-evasive mechanisms mediated by the PD-1/PD-L1 pathway; hence, simultaneous inhibition of TGFβ and PD-L1 pathways may allow for increased overall efficacy compared with independent blockade of either pathway alone. Results from various preclinical models have suggested that the bifunctional blockade of the PD-1/PD-L1 and TGFβ pathways offers a unique approach to

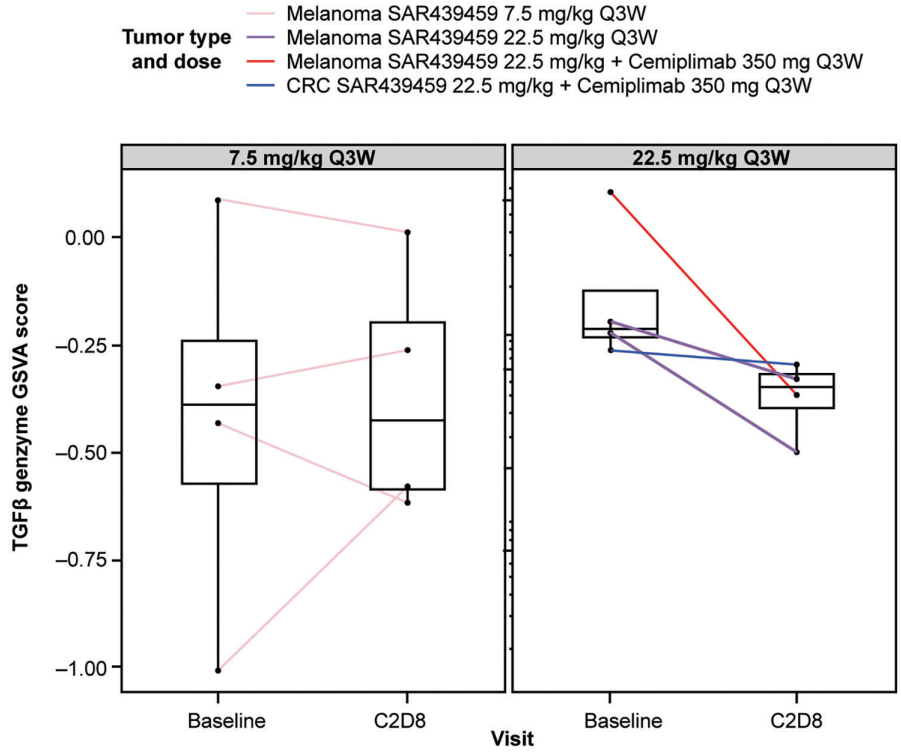

FIGURE 2 Modulation of transforming growth factor-beta (TGFβ) pathway activation signature in tumor tissue. Representative box plots showing the TGFβ gene expression ($N=4$ patients in each SAR439459 7.5 mg/kg Q3W and SAR439459 22.5 mg/kg Q3W). Colored lines indicate individual tumor biopsy samples. C2D8, Cycle 2 Day 8; CRC, colorectal cancer; GSVA, gene set variation analysis; Q3W, every 3 weeks; TGFβ, transforming growth factor-beta.

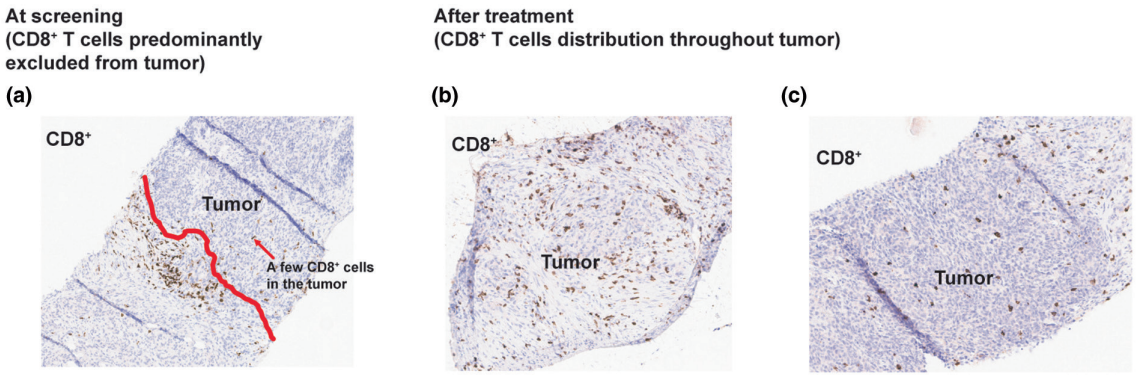

FIGURE 3 $CD8^+$ staining of specimens from a patient with melanoma (a) before and (b, c) after treatment with SAR439459 22.5 mg/kg + cemiplimab 350 mg Q3W. Representative tissue section showing $CD8^+$ T cells. The red line indicates the tumor border. Q3W, every 3 weeks.

engaging the immune system and promoting antitumor efficacy.[5–7] However, to date there are limited clinical data to confirm whether this mechanism could translate to patients. The clinical data from the phase I/Ib study of SAR439459, alone and in combination with anti-PD-1, were recently presented. However, the study was discontinued due to lack of sufficient antitumor response and the observed bleeding risk particularly in the HCC cohort.[13] Herein, PD and patient enrichment biomarker data from this first-in-human study are presented to enhance our understanding of peripheral and tumor PD effects of SAR439459.

Target engagement of SAR439459, as monotherapy or in combination with anti-PD-1, was documented from the first dose level in the blood and at the pRP2D in the limited paired tumor samples, by modulation of TGFβ levels and the related signaling pathway. Previously, we have shown that in escalation cohorts TGFβ levels were

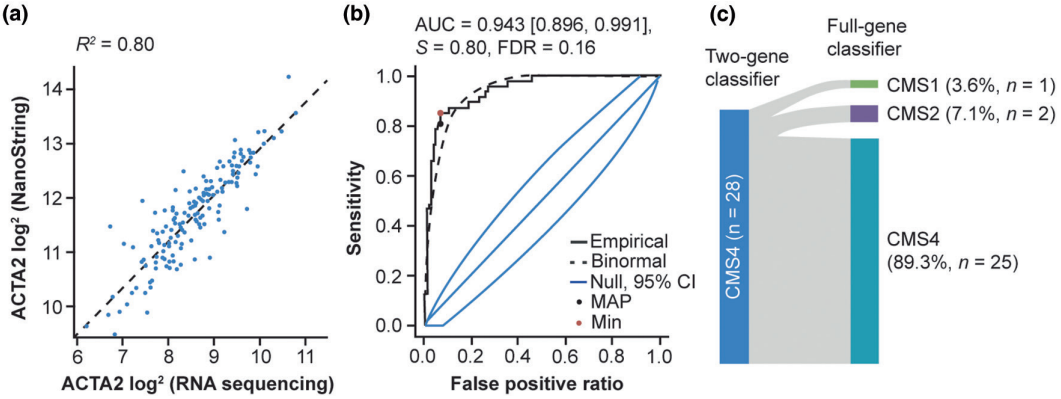

FIGURE 4 (a) Comparison of ACTA2 gene expression between NanoString and RNA sequencing in colorectal cancer (CRC) tumor samples; (b) sensitivity versus false-positive ratio in CRC tumor samples; (c) CMS4 classification using two-gene classifier and full epithelial to mesenchymal transition (EMT)-gene classifier methodologies. ACTA, actin A; AUC, area under the curve; CI, confidence interval; CMS, consensus molecular subtype; CRC, colorectal cancer; FDR, false discovery rate; MAP, define; R^2, coefficient of determination.

down-regulated robustly from the lowest dose level (0.05 mg/kg) right after the administration.[10] In addition, here we also demonstrated the data from escalation cohorts that a trend of increase in key immune cells, including CD8 and NK cells, was observed in blood; however, no significant PD change has been confirmed (**Figure S2**). This immune cell modulation is accompanied with Th1 cytokine, such as CXCL-10, that is consistent with our preclinical observation.[5,10] Further analysis confirmed observing trend of peripheral T cell and NK cell proliferation and activation in patients treated with SAR439459 with or without cemiplimab, although the modulation was not significant. In addition, the number of $CD8^+$ T cells in the tumor microenvironment post-treatment with SAR439359 alone and in combination with cemiplimab was greater than that observed at baseline (**Figure S3**). The impact of SAR439459 on peripheral PD biomarkers, coupled with $CD8^+$ T cell modulation in the tumor microenvironment, is consistent with TGFβ signaling inhibition. While this altogether led to the overcoming of immune exclusion in some cases, it was with significant variability and did not translate to relevant antitumor activity. Taken together, SAR439459, alone or in combination with cemiplimab, induced T cell and NK cell proliferation and activation in the blood. The impact of SAR439459 on peripheral PD biomarkers, coupled with $CD8^+$ T cell modulation in the tumor microenvironment, is consistent with the mode of action from TGFβ blockade.

CMS4 is one of the four consensus molecular subtypes of CRC and is marked by the activation of the TGFβ pathway. TGFβ is a potent inducer of EMT, which contributes to the aggressive nature of the CMS4 mesenchymal phenotype, leading to increased invasion, migration, and metastasis. In addition, the TGFβ pathway contributes to immune evasion in CMS4 tumors by suppressing the activity of immune cells, such as T cells, and promoting the expansion of immunosuppressive cells, such as Tregs and myeloid-derived suppressor cells. This immune suppression allows the tumor to evade immune surveillance and continue growing unchecked. Therefore, targeting the TGFβ pathway may represent a promising therapeutic approach for patients who have tumors with the CMS4 mesenchymal phenotype. To identify patients with CMS4 mesenchymal phenotype tumors, a two-gene classifier was developed and implemented in a diagnostic Clinical Laboratory Improvement Amendments (CLIA)-certified laboratory. The overall performance and specificity of this two-gene classifier was particularly good. The area under the curve (AUC) of the classifier is 0.943 and 95% confidence interval for the AUC is [0.896, 0.991].

Given the complex role of TGFβ in cancer, it is essential to identify patients who are most likely to respond to the therapy so as to maximize the benefit of TGFβ blockade. One potential strategy is measuring the TGFβ protein levels in tumor tissues or blood samples and then selecting patients who have elevated TGFβ levels as this may indicate that TGFβ signaling is actively driving tumor progression in these individuals. Therefore, in the present study, highly specific assays were developed and then used to measure active TGFβ1, TGFβ2, and TGFβ3 in a substantial number of flash-frozen NSCLC tumors along with total TGFβ1 and TGFβ2 in paired plasma samples from each respective patient aiming to evaluate the distribution of TGFβ levels. It was found that active and total TGFβ1 were more consistently expressed in the procured NSCLC patient samples followed by TGFβ2, whereas TGFβ3 was only measurable (LLOQ ≥2.68 pg/mg) in tumors. Distribution and prevalence analysis of TGFβ1 in tumor and plasma identified various cutoff values, which may provide insight for patient stratification and selection threshold identification. The observation in this study also confirmed that

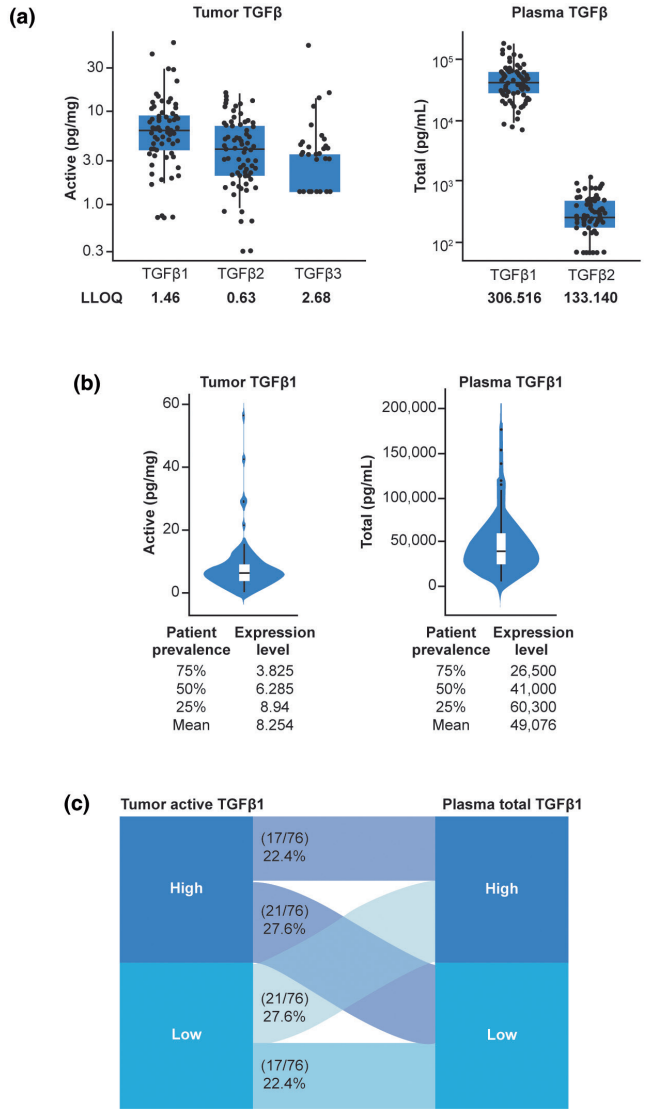

FIGURE 5 (a) Transforming growth factor-beta (TGFβ1) expression in plasma and tumor; (b) concordance analysis between intratumoral active and plasma total TGFβ1 levels; and (c) prevalence and distribution of TGFβ1. Expression level of TGFβ isoforms in periphery and tumor. (a) Box and whisker plot distribution of active TGFβ1, TGFβ2, and TGFβ3 isoforms in fresh frozen non-small cell lung carcinoma tumor samples. Lower limit of quantitation (LLOQ) for each assay is indicated below each plot, and individual values below the LLOQ are imputed as a value of half of the respective LLOQ. (b) Box and whisker plot distribution of total (active and latency-associated peptide [LAP] TGFβ) TGFβ1 and TGFβ2 isoforms in the paired plasma samples. The LLOQ for each assay is indicated below each plot. (c) The concordance between tumor and plasma TGFβ1 values of 76 paired samples is illustrated by the Sankey plot representing the number of samples in each category and the representative percentage of the whole. Samples are stratified by median into a high subgroup (2 median) and a low subgroup (<median) for both tumor and plasma TGFβ1. Cohen's kappa was performed to measure the concordance, and no agreement between tumor active TGFβ1 and plasma total TGFβ1 stratified by median was noted. LLOQ, lower limit of quantitation; TGFβ, transforming growth factor-beta.

accurate and reliable measurement of TGFβ protein levels can be technically challenging and thus standardized assays need to be developed for consistent results across various laboratories in multicentered clinical studies. Efforts were also made to correlate intratumoral TGFβ levels to those found in the periphery (plasma) with the goal to assess the predictive value of plasma TGFβ levels as a potential surrogate to intratumoral TGFβ expression in the selection of patients with high TGFβ levels; however, only a small group of samples demonstrated the association, suggesting the difficulty in using plasma TGFβ as a surrogate of intratumoral TGFβ level. Using TGFβ protein levels to select patients who may benefit from TGFβ blockade is a promising approach; however, further clinical research is needed to validate this strategy in larger and well-designed clinical studies to establish the correlation to patient outcome.

Collecting tumor and blood samples for biomarker data analysis plays a crucial role in early clinical studies, which contributes to a deeper understanding of mode of action and evaluating the efficacy and safety of a new treatment. In the present study, the valuable patient blood and tumor biopsy samples allowed us to perform detailed molecular and genetic analysis, which is crucial for confirming the target engagement, understanding the immune cell modulation, and monitoring the TGFβ gene pathway modulation. In addition, the gene classifier identified the patient population with a tumor CMS4 phenotype that was predicted to benefit most likely from TGFβ inhibitor treatment and to enhance treatment efficacy and minimize unnecessary side effects. Biomarker assessment plays an important role in refining clinical design strategies, enhancing patient safety, and improving the chances of clinical success. Therefore, the assessment of biomarkers in early clinical trials is critical to the modern oncology drug development journey.

CONCLUSIONS

In conclusion, the present study provided a systemic summary of peripheral and tumor PD results as well as the cancer patient enrichment strategies from the phase I/Ib trial of SAR439459, as monotherapy and in combination with cemiplimab, in patients with advanced solid tumors. The study demonstrated that while SAR439459, when combined with anti-PD-1 therapy, elicited the expected peripheral PD effects in peripheral blood, there is inadequate evidence of induced $CD8^+$ T cell infiltration in the tumor microenvironment upon treatment with SAR439459 due to limited sample size and intratumoral heterogeneity. The current biomarker data are complementary to this recent publication on the clinical activity of SAR439459 alone or in combination with

cemiplimab in an unselected population of advanced solid tumors,[13] and these data contribute to our understanding of the lack of enough therapeutic benefit from the treatment and the potential patient enrichment strategies to future clinical studies on TGFβ blockade therapy in cancer patients. However, this study was limited due to the small sample size, especially the paired tumor biopsy, from a homogeneous population to confirm the dose-dependent target engagement and PD effects in the tumor microenvironment. The small sample size further limited the statistical power to evaluate the association between the biomarkers and clinical response, thus resulting in the lack of identification of the efficacy biomarker from the current study. Nonetheless, SAR439459 has shown promising responses in combination with cemiplimab in some patients refractory to immune checkpoint inhibitors; however, further studies are needed to identify biomarkers of response.

AUTHOR CONTRIBUTIONS

All authors wrote the manuscript, designed the research, performed the research, and analyzed the data.

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CONFLICT OF INTEREST STATEMENT

J.-J.G.: Consulting or Advisory Board – BMS, MSD Oncology, Roche/Genentech, Novartis, Amgen, Pierre Fabre, Merck KGaA, Sun Pharma, Sanofi, Roche, Philogen, Ultmovacs; Speakers' Bureau – Novartis; Travel, Accommodation, Expenses – Bristol-Myers Squibb, USD Oncology, Novartis, Pierre Fabre. O.B.: Advisory Board – Sanofi. M.O.B.: Advisory Board – Regeneron, Ideaya Bio; Honoraria – Sanofi, Pfizer; Grant – Novartis; Others – Adaptimmune, GSK. D.R., B.D., I.B., M.D.N., M.S., A.R.V., T.C.: Nothing to disclose. P.M.R.: Grants – AstraZeneca; Current Employment and Stock/Stock Options – AstraZeneca/Medimmune. E.P.–T.: Travel – AstraZeneca, Pfizer; Advisory Board – AstraZeneca, Sanofi, Takeda, Bristol-Myers Squibb. G.C.: Grants – Merck; Consulting Fees – Bristol-Myers Squibb, Roche, Pfizer, Novartis, Lilly, Astra Zeneca, Daichii Sankyo, Merck, Seagen, Ellipsis, Gilead; Honoraria – Eli-Lilly, Pfizer, Relay; Travel – Daichii Sankyo. M.-H.R.: Consulting Fees – Daiichi Sankyo, AstraZeneca, ONO, Bristol-Myers Squibb, MSD, Taiho, Novartis, Daehwa Pharmaceutical; Honoraria – Sanofi, Daiichi Sankyo, AstraZeneca, ONO, Bristol-Myers Squibb, MSD, Taiho, Novartis, Daehwa Pharmaceutical. D.S.: Grants – Roche, Bristol-Myers Squibb, MSD, Novartis, Amgen, Pfizer; Others – Roche, Bristol-Myers Squibb, MSD, Novartis, Pierre Fabre, Nektar, Regeneron, Sanofi, Replimune, Philogen, Neracre, Sun Pharma; Personal Fees – Bristol-Myers Squibb, MSD, Novartis, Pierre Fabre, Nektar, Regeneron, Sanofi, Replimune, Pfizer, Philogen, Neracre, Sun Pharma, Daiichi Sankyo, Immatics, Ultimovacs; Non-Financial Support – MSD, Novartis, Pierre Fabre, Nektar, Regeneron, Sanofi, Replimune. E.G.: Consulting Fees – Roche/Genentech, F. Hoffmann-La Roche, Ellipses Pharma, Neomed Therapeutics 1 Inc., Boehringer Ingelheim, Janssen Global Services, Seagen, TFS, Alkermes, Thermo Fisher,

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DATA AVAILABILITY STATEMENT

Qualified researchers may request access to patient-level data and related documents (including, e.g., the clinical study report, study protocol with any amendments, blank case report form, statistical analysis plan, and dataset specifications). Patient-level data will be anonymized, and study documents will be redacted to protect the privacy of trial participants. Further details on Sanofi's data-sharing criteria, eligible studies, and process for requesting access can be found at https://vivli.org/.

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REFERENCES

1. Kim BG, Malek E, Choi SH, Ignatz-Hoover JJ, Driscoll JJ. Novel therapies emerging in oncology to target the TGF-β pathway. *J Hematol Oncol*. 2021;14:55. doi:10.1186/s13045-021-01053-x
2. Neuzillet C, Tijeras-Raballand A, Cohen R, et al. Targeting the TGFβ pathway for cancer therapy. *Pharmacol Ther*. 2015;147:22-31. doi:10.1016/j.pharmthera.2014.11.001
3. Gregory RC, Greco R, Qu H, et al. The anti-TGFβ neutralizing antibody, SAR439459, blocks the immunosuppressive effects of TGFβ and inhibits the growth of syngeneic tumors in combination with anti-PD1. *Cancer Res*. 2018;78:2790. doi:10.1158/1538-7445.AM2018-2790
4. Akhurst RJ, Hata A. Targeting the TGFβ signalling pathway in disease. *Nat Rev Drug Discov*. 2012;11:790-811. doi:10.1038/nrd3810
5. Greco R, Qu H, Qu H, et al. Pan-TGFβ inhibition by SAR439459 relieves immunosuppression and improves antitumor efficacy of PD-1 blockade. *Onco Targets Ther*. 2020;9:1811605. doi:10.1080/2162402x.2020.1811605
6. Lan Y, Zhang D, Xu C, et al. Enhanced preclinical antitumor activity of M7824, a bifunctional fusion protein simultaneously targeting PD-L1 and TGF-β. *Sci Transl Med*. 2018;10:eaan5488. doi:10.1126/scitranslmed.aan5488
7. Mariathasan S, Turley SJ, Nickles D, et al. TGFβ attenuates tumour response to PD-L1 blockade by contributing to exclusion of T cells. *Nature*. 2018;554:544-548. doi:10.1038/nature25501
8. Tauriello DVF, Palomo-Ponce S, Stork D, et al. TGFβ drives immune evasion in genetically reconstituted colon cancer metastasis. *Nature*. 2018;554:538-543. doi:10.1038/nature25492
9. Cemiplimab Package Insert. Accessed January 06, 2023. https://www.accessdata.fda.gov/drugsatfda_docs/label/2022/761097s014lbl.pdf
10. Williamson S, Hodi SF, Johnson M, et al. Safety, pharmacokinetic and pharmacodynamic results from dose escalation of SAR439459, a TGFβ inhibitor, as monotherapy or in combination with cemiplimab in a phase 1/1b study (Abstract 2510). *J Clin Oncol*. 2021;39(15_suppl):2510. doi:10.1200/JCO.2021.39.15_suppl.2510
11. Baron U, Werner J, Schildknecht K, et al. Epigenetic immune cell counting in human blood samples for immunodiagnostics. *Sci Transl Med*. 2018;10:eaan3508. doi:10.1126/scitranslmed.aan3508
12. Theilhaber J, Cavallo J, Madden SL, et al. Translational biomarkers for SAR439459, an anti-TGFβ antibody for cancer immunotherapy. *Cancer Res*. 2018;78:5550. doi:10.1158/1538-7445.AM2018-5550
13. Robbrecht D, Doger B, Grob J-J, et al. Safety and efficacy results from the expansion phase of the first-in-human study evaluating TGFβ inhibitor SAR439459 alone and combined with cemiplimab in adults with advanced solid tumors. *J Clin Oncol*. 2022;40:2524. doi:10.1200/JCO.2022.40.16_suppl.2524

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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