Stool Bacteriomic Profiling in Patients with Metastatic Renal Cell Carcinoma Receiving Vascular Endothelial Growth Factor–Tyrosine Kinase Inhibitors

Sumanta K. Pal, Sierra M. Li, Xiwei Wu, Hanjun Qin, Marcin Kortylewski, JoAnn Hsu, Courtney Carmichael, and Paul Frankel

Abstract

Purpose: Diarrhea occurs in approximately half of patients with metastatic renal cell carcinoma (mRCC) receiving vascular endothelial growth factor–tyrosine kinase inhibitors (VEGF-TKIs). We evaluated the relationship between VEGF-TKI–related diarrhea and stool microbiota.

Experimental Design: Stool samples were collected from 20 mRCC patients receiving VEGF-TKIs. 16S rRNA sequencing was used to characterize the stool bacteriomic profiling of patients. Assay validation with Salmonella typhimurium spike-in experiments suggested greatest speciation with use of the V5 region.

Results: Higher levels of Bacteroides spp. and lower levels of Prevotella spp. were found in patients with diarrhea. In addition, patients receiving VEGF-TKIs with mRCC appeared to have less relative abundance of Bifidobacterium spp. as compared with previous reports based on healthy subjects.

Conclusions: We have thus demonstrated interplay between microbiota and VEGF-TKI–induced diarrhea. Further studies are warranted to evaluate the potential causative role of preexisting dysbiosis in VEGF-TKI–related diarrhea. Clin Cancer Res; 1–8. ©2015 AACR.

Introduction

The systemic therapy of metastatic renal cell carcinoma (mRCC) has evolved markedly over the past decade with the advent of targeted treatments. Approved treatments for this disease fall into two mechanistic categories: (i) inhibitors of VEGF and its cognate receptor, and (ii) inhibitors of the mTOR (1). The first category is composed of five agents—one monoclonal antibody (bevacizumab) and four small-molecule VEGF-tyrosine kinase inhibitors (VEGF-TKI; sunitinib, sorafenib, pazopanib, and axitinib). Two mTOR inhibitors are currently approved, everolimus and temsirolimus.

In the first-line setting, patterns of care data suggest that the preponderance of patients receive VEGF-TKIs (2). VEGF-TKIs include hypertension, fatigue, hand-foot syndrome, nausea, and diarrhea (3). A challenge in daily practice is that few evidence-based strategies exist to combat these toxicities. Although several consensus statements exist about the management of VEGF-TKI–related toxicity, the recommendations offered therein are typically supported by anecdotal evidence or clinical experience (4).

The underlying aim of the current study was to better define the biologic basis of diarrhea associated with VEGF-TKIs. Across the pivotal studies of first-line VEGF-TKIs, the cumulative incidence of all grade diarrhea is 51%, and the cumulative incidence of grade 3/4 diarrhea is 10% (5–7). The etiology of VEGF-TKI–related diarrhea is poorly understood. It has been postulated that these agents may cause direct damage to colonic mucosa. As these agents may have a profound systemic immune effect, it has been suggested that diarrhea could potentially be inflammatory in nature (8–10). The role of gut microbiota in immune-related inflammation [e.g., through lipopolysaccharides (LPS) or fermentation of butyrates in colonocytes] provides motivation to investigate the relationship between the microbiota and VEGF-TKI–induced diarrhea (11, 12). As a first step, we focus on demonstrating that patients with VEGF-TKI–induced diarrhea have a different microbiota population than patients without diarrhea. This approach does not address whether the microbiota are reacting to the diarrhea or whether the different microbiota populations permit or cause diarrhea, but it provides incentive for more definitive studies and allows us a first examination of microbiota in mRCC patients receiving VEGF-TKIs. This also provides an opportunity to explore the phylogenetic abundance of specific microbial populations in the gut that may be associated with VEGF-TKI–induced diarrhea.
Translational Relevance

Diarrhea occurs in roughly 50% patients with metastatic renal cell carcinoma (mRCC) receiving vascular endothelial growth factor–tyrosine kinase inhibitors (VEGF-TKIs). To date, the etiology of VEGF-TKI–related diarrhea has not been determined, nor have evidence-based strategies been devised to manage this toxicity. We report differences in stool bacterial profile (assessed via 16S RNA sequencing) in mRCC patients with and without diarrhea while on VEGF-TKI therapy. Higher levels of Bacteroides spp. and lower levels of Prevotella spp. were found in patients with diarrhea. Further validation of our findings could lead to probiotic regimens (e.g., regimens fortified with Prevotella spp.) that could be used to ameliorate diarrhea in this population. Conversely, an alternative could be elimination of potentially pathogenic species (e.g., Bacteroides spp.).

Patient selection

Given the small anticipated sample size in this pilot study, a dichotomous variable. Patients noted as having diarrhea or the absence of diarrhea was characterized as a dichotomous. Diarrhea occurs in roughly 50% patients with metastatic renal cell carcinoma (mRCC) receiving vascular endothelial growth factor–tyrosine kinase inhibitors (VEGF-TKIs). To date, the etiology of VEGF-TKI–related diarrhea has not been determined, nor have evidence-based strategies been devised to manage this toxicity. We report differences in stool bacterial profile (assessed via 16S RNA sequencing) in mRCC patients with and without diarrhea while on VEGF-TKI therapy. Higher levels of Bacteroides spp. and lower levels of Prevotella spp. were found in patients with diarrhea. Further validation of our findings could lead to probiotic regimens (e.g., regimens fortified with Prevotella spp.) that could be used to ameliorate diarrhea in this population. Conversely, an alternative could be elimination of potentially pathogenic species (e.g., Bacteroides spp.).

Materials and Methods

Patient selection

Key eligibility for the study included histologically confirmed RCC, distant metastatic disease, and current therapy with one of four VEGF-TKIs (sunitinib, sorafenib, pazopanib, or axitinib). Patients receiving investigational VEGF-TKIs or combination therapy were excluded from participation. Patients had to demonstrate an understanding of stool collection and transport procedures and be willing to comply with these procedures. In this pilot study, the timepoint of stool collection was not standardized, but patients were approached for consent after at least 2 weeks of therapy with VEGF-TKI.

Both the protocol and informed consent were approved by the institutional scientific review committee, data safety monitoring board, and the Institutional Review Board. All patients enrolled provided written informed consent, and the study was conducted in accordance with the amended Declaration of Helsinki and the International Conference on Harmonization Guidelines.

Characterization of diarrhea

Patients were characterized by the principal investigator (S.K. Pal) as having grade 1–4 diarrhea (using Common Toxicity Criteria for Adverse Events; CTCAE; 4.0) or having no diarrhea. Given the small anticipated sample size in this pilot study, a specific CTCAE grade was not assigned to the patient; rather, the presence or absence of diarrhea was characterized as a dichotomous variable. Patients noted as having diarrhea or the absence of diarrhea submitted stools specimens while reporting these symptoms or absence of symptoms, respectively. Only a single sample was obtained from each patient.

Specimen collection

A standard operating procedure document was generated and distributed to patients. Briefly, a random stool specimen was collected by the patient and placed in a sealed plastic container immediately. The sealed plastic container was wrapped in a cooling pack (maintaining a temperature of roughly 4°C). The container and cooling pack were then placed in an insulated foam container and shipped overnight to the City of Hope Integrated Genomics core within 24 hours.

Spike-in experiment using V3, V4, and V5 regions

To evaluate the performance of bacterial classification, we performed a spike-in analysis using a laboratory strain of Salmonella typhimurium (S.Tm 700720 Cat #700720D-5, ATCC) included in Greengene database under OTU_3620. A fecal sample from a healthy donor was used for this experiment. S. typhimurium alone was used as a positive control and its DNA was mixed with the fecal DNA at levels of 1%, 5%, 25%, and 75%. Illumina sequencing was performed for all three regions with paired ends. QIME was used for filtering quality reads and closed-reference approach with the UCLUST and USEARCH algorithms to classify reads to OTUs in Greengene database. The details of sequencing and OTU classification are described subsequently. USEARCH is a more updated version of UCLUST algorithm and appears to be superior in bacterial classification across all three regions. The V5 region with the USEARCH algorithm had the best accuracy in identifying S. typhimurium at the species level. The V5 region outperformed the V3 and V4 regions in accuracy. For the true spike-in level of Salmonella at 1%, 5%, 25%, and 75%, the estimated levels using the V5 region and USEARCH algorithm were 0.9%, 4.2%, 18.4%, and 66.5% respectively. In comparison, the region more commonly used based on theoretical coverage considerations, V4, returned levels of 1.1%, 5.2%, 21.3%, and 67.3%, respectively, but it failed to assign the spike-in bacteria to the correct genus and species.

Fecal DNA isolation and multiplex sequencing

Total genomic DNA was isolated from 0.25 g of feces using the PowerSoil DNA isolation kit (Mo Bio). Purified DNA was separated on a 1% agarose gel and quantified by densitometry and spectrophotometry (NanoDrop 1000; Thermo Scientific). Because the V5 region in the spike-in analysis performed better than V3 and V4, all fecal samples from patients were sequenced using V5 regions. As described by Stearns and colleagues, a PCR protocol was used to amplify bacterial 16S rRNA genes from all samples (17). The following PCR primers (including Illumina part of adapter sequences) were used to amplify V5 region:

V5-F: ACACCTCTTCCCTACGGGAGGCAGCAG
V5-R: GTGACTGAGTTCAGACGTGTGCTCTTCCGATCTCAATTCMTTTGAGTT.

Complete Illumina adapter and barcodes were added by another five cycles of PCR to make an Illumina library. After bioanalyzer and qPCR checking for quality control, multiple libraries were mixed equally. Paired-end of sequencing (2 × 100 bp) was performed by Illumina HiSeq 2000.
Analysis of fecal microbiota

Software QIIME was used to analyze the Illumina sequencing reads generated for the V5 region of 16S rRNA (18). The paired end reads were first joined by fastq-join to generate longer reads with median length varying from 138 bp to 189 bp among the patient samples. Default QIIME quality filters were applied, including the maximum number of consecutive low-quality base calls allowed and minimum number of consecutive high-quality base calls to include a read (19). Chimera sequences generated by PCR amplification of multiple templates or parent sequences were identified by ChimeraSlayer and subsequently filtered out before further analysis (20). Operational taxonomic units (OTU) for 16S rRNA were picked from Illumina reads using a closed-reference OTU picking protocol (see Supplementary Appendix 1). A table of OTU counts per sample was generated and the abundance was summarized for OTU with/C21r ecord phyllum, class, order, family, genus, and species levels. The relative abundance was defined as the percentage of reads mapped to the specific OTU. Unifrac distances between samples were calculated using the Greengene reference tree (21). The table of the OTU counts per sample was used in the combination of tree structures from Greengene database to generate α and β diversity.

Clustering analysis

Clustering analysis was performed with 16S rRNA OTU relative abundance tables at the genus and species levels. We considered several distance matrices including Jensen–Shannon divergence (JSD) and weighted/un-weighted UniFrac distances. Clustering analysis was carried out by partitioning around medoids (PAM), a more robust version of “K-means” clustering. This minimized the sum of dissimilarities based on distance matrix (22). The Calinski–Harabasz (CH) index was used to assess the optimal number of clusters, whereas the Silhouette index validated the cluster. The Silhouette index for each sample varied from 0 to 1—a larger value (~1) indicated that the sample was well clustered and a small value closer to 0 indicated that the sample was between 2 clusters. The major principle coordinates (PC) from PCoA analysis were studied to show a possible correlation with the presence of diarrhea and other covariates such as type of drugs and line of treatment.

Results

Patient characteristics

Patient characteristics are summarized in Table 1. A total of 20 patients were consented and enrolled. Of these, 12 patients had diarrhea with VEGF-TKI therapy and 8 did not. The majority of patients enrolled were male (60%), and the median age at diagnosis was 63. The most frequent sites of metastases (in descending order) were lung, liver, and bone. The majority of patients (60%) had two or more prior therapies, whereas the remainder had no prior treatment. Sunitinib was the most frequently utilized VEGF-TKI (55%), followed by pazopanib (25%), axitinib (10%), and sorafenib (10%). Because of the limited number of patients receiving agents other than sunitinib, comparisons hereafter are made between sunitinib and non-sunitinib–treated patients. Patients were on VEGF-directed treatment for a median of 15.2 months (range, 0.5–46) before collection of stool specimens for the current analysis. Notably, on retrospective review of patient medical records, no patients had a documented history of inflammatory bowel disease or other gastrointestinal illness that would predispose to diarrhea. All patients were on standard doses of VEGF-TKIs (e.g., sunitinib at 50 mg oral daily, axitinib at 5 mg oral twice daily, sorafenib at 400 mg oral twice daily).

Table 1. Clinical and pathologic characteristics

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<td>12 (60)</td>
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<tr>
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<td>8 (40)</td>
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<tr>
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<td>6 (75)</td>
<td>9 (75)</td>
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<tr>
<td>Poor</td>
<td>5 (25)</td>
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<td>5 (63)</td>
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</tr>
<tr>
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<td></td>
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<td></td>
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<tr>
<td>Sunitinib</td>
<td>11 (55)</td>
<td>4 (49)</td>
<td>7 (59)</td>
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<td>Pazopanib</td>
<td>5 (25)</td>
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<td>3 (25)</td>
</tr>
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<td>2 (10)</td>
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<td>1 (8)</td>
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<tr>
<td>Sorafenib</td>
<td>2 (10)</td>
<td>1 (13)</td>
<td>1 (8)</td>
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</table>
daily, or pazopanib at 800 mg oral daily); no patients had dose reductions before stool collection. Furthermore, no patients had diarrhea preceding initiation of their current VEGF-directed therapy.

**Bacterial abundance and diversity**

DNA was extracted from a single fecal sample from each of the 20 patients. The V5 region of the 16S rRNA genes presented in the DNA was amplified by PCR and resulting amplicons were

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**Figure 1.**

A, average relative abundance across samples at the genus level for the 15 most abundant genera. Genera are colored by their respective phylum. The top right subplot shows the abundance level at the phylum level. B, average relative abundance level at the species level for the 15 most abundant species. OTUs with unclassified genera or species were removed and relative abundance levels were restandardized.
sequenced on an Illumina HiSeq 2000. A total of 27,170,454 paired-end reads were generated for the 20 patients with an average of 1,086,818 reads (SD 302,304) per patient. About 90.9% of the raw reads were able to be joined by the two ends and 91.6% of the joint reads passed the QIIME quality default quality filter. Furthermore, we applied a Chimera sequence filter and removed an additional 0.12% of reads, retaining about 83.2% of the original raw reads for final analysis. Notably, 98.7% of sequences matched reference sequences in the Green-gene database with ≥97% similarity. Counts for each OTU identified were tabulated across samples and the relative abundance are summarized at phylum, class, order, family, genus, and species levels. As shown in Fig. 1, the most prominent phyla were Bacteroidetes (67.91%) and Firmicutes (26.20%), which were significantly more abundant than Proteobacteria (2.04%) and Actinobacteria (0.08%). At the genus level, the most prominent five genera made up 54.93% of the abundance. The two genera from phylum Bacteroidetes were Bacteroides (47.37%) and Tannerella (0.84%). The remaining genera were Faecalibacterium (3.75%), Ruminococcus (1.88%), and Blautia (1.09%) from phylum Firmicutes. We noticed that the gut microbiota of patients treated with a VEGF-TKI was very different from that of subjects who did not undergo drug treatment, based on a previously published report from Arumugam and colleagues (23). Most notably, Bifidobacterium was the third most abundant genus present in the cited study, whereas Bifidobacterium was not in the top 15 genera in our cohort. In fact, Bifidobacterium was often undetected, with only 2 of the 8 patients without diarrhea and 2 of the 12 with diarrhea with detectable levels ≥0.1%.

Alpha diversity was calculated to show species abundance. Samples were rarefied to 419,950 reads per sample. As seen in Fig. 2 and Supplementary Fig. S1, both Shannon and Simpson indices revealed that there is no obvious difference in α diversity between diarrhea and non-diarrhea patients, though the α diversity of non-diarrhea patients was slightly less than average. In addition, there was no distinctive correlation between α diversity and age in patients 60 to 70 years old. However, we noticed that there were 3 patients over 75 with much lower α diversity compared with other patients. We determined there was no statistically significant difference for Shannon index between diarrhea status, first-line treatment versus others, and sunitinib versus other drugs. Furthermore, no statistically significant association was seen between Shannon index and time on VEGF-TKI therapy (Supplementary Fig. S2a).

**Cluster analysis and PCoA**

QIIME identified 247 genera but some had very low relative abundance. We reduced the noise signal by removing genera with average abundance <0.01% across the 20 patients and performed clustering analysis with PAM for the remaining 80 genera. JSD was used for calculating dissimilarity. The patient overall cohort appeared to be very heterogeneous in gut microbiome. We ultimately clustered the 20 patients at the genus level with two clusters. Because of the high level of heterogeneity, the silhouette

![Figure 2](https://example.com/figure2.png)

**Figure 2.** Shannon index plotted against (A) age, (B) diarrhea status, (C) first-line therapy versus others, and (D) sunitinib versus other systemic treatment.
index was 0.23. We used PCoA to interpret and visualize the two clusters identified by PAM. Of the cohort, 12 patients developed diarrhea and 8 did not develop diarrhea. The first cluster, termed the "high-risk" group, included 11 diarrhea patients and 4 non-diarrhea patients, whereas the second cluster, the "low risk" group, contained 4 non-diarrhea patients and 1 diarrhea patient. Fisher exact test was used to determine whether there was a nonrandom association between the clusters and diarrhea status. Although we obtained a small $P$ value of 0.10 and the clusters appeared to be biologically meaningful, further study with a larger sample size is necessary to confirm these clusters.

The separation of the two groups is clear in Fig. 2A when plotting the first two PCs. To further investigate which genera were the driving factors for the two clusters, we used a t test to compare the difference in average abundance between the two clusters. Bacteroides and Prevotella were the two genera with distinct abundance ($P < 0.05$ for both) and an absolute difference $\geq 20\%$ (Supplementary Table S1). As shown in Fig. 3B, the high-risk group appeared to have a greater abundance of Bacteroides (42%) and a very low level of Prevotella (3%). The low-risk group displayed the opposite pattern with high levels of Prevotella (47%) and low levels of Bacteroides (13%).

We evaluated the association between the first two principal coordinates with age, diarrhea status, first-line therapy, and use of sunitinib. As shown in Supplementary Fig. S3, PC1 appears to be associated with diarrhea status, despite $P$ value not being significant ($P = 0.14$). PC2 was marginally associated with first-line treatment compared with other line of treatment ($P = 0.08$).

Similar cluster analysis was also performed at the species level. QIIME identified 346 species. After removing species with low abundance ($\leq 0.01\%$), 88 species were left for clustering. Similarly, two clusters were obtained at the species level. The high-risk group included 10 diarrhea patients and 3 non-diarrhea patients, whereas the low-risk group contained 5 non-diarrhea patients and 2 diarrhea patients. The two species driving the clustering shown in Fig. 3 were an unclassified Bacteroides species and Prevotella copri. The pattern for abundance at species level was very similar to the pattern shown at the genus level in Fig. 2B. Notably, abundance of Bacteroides and Prevotella was not significantly associated with duration of VEGF-TKI therapy (Supplementary Fig. S2b and S2c).
Comparison between diarrhea and non-diarrhea patients

There were 247 and 346 OTUs identified at the genus and species level, including those that were unclassified. After data denoising, we removed OTUs with average abundance 0.01% or less, leaving 71 genera and 88 species for the subsequent test. Supplementary Table S2 lists the average difference bacterial abundance measured by percentage for 10 OTUs with smallest P values. Five genera and five species showed a significant difference in abundance (P ≤ 0.05). However, the difference in abundance appeared to be marginal with a range of 0.01% to 0.25%. In contrast, the t test between the two clusters obtained by PAM and JSD distance revealed large differences in bacterial species (Fig. 3 and Supplementary Fig. S4 and Supplementary Table S1). At the genus level, the average differences between the high-risk and low-risk clusters were 32.55% and 42.89% for Bacteroides and Prevotella with P values of 0.001 and 0.004, respectively. At the species level, the average differences between the two clusters were 26.10%, 14.61%, and 24.12% for an unclassified species under Bacteroides, Bacteroides acidifaciens, and Prevotella copri with P value <0.001, 0.002, and 0.04, respectively. The high-risk and low-risk groups appeared to reveal biologically meaningful information on bacterial abundance with between-group differences (Fig. 3).

Discussion

To our knowledge, this is the first effort to characterize the stool bacteriomic profile of patients with mRCC receiving VEGF-TKIs. Given the incidence of gastrointestinal toxicity associated with VEGF-TKIs, it was hypothesized that stool flora may be associated with the presence or absence of diarrhea. Our results support a positive and negative association between Bacteroides spp. and Prevotella spp. and diarrhea, respectively. At present, treatment recommendations for VEGF-TKI–related diarrhea include use of anti-motility agents, which do little to combat the underlying mechanism of diarrhea (4). Although recommendations for probiotic supplementation have been offered based on anecdotal clinical experience, the utility of this strategy has not been formally assessed. Further validation of our work may potentially offer an evidence-based approach to treating VEGF-TKI–related diarrhea.

Our findings are supported by preclinical work, suggesting that an increase in Bacteroides spp. accompanies the onset of chemotherapy-induced diarrhea in rodent models (24, 25). Bacteroides spp. represents potentially enteropathogenic species, and therefore, their association with the higher-risk group for diarrhea is logical (26). Also consistent with our findings, Prevotella spp. has been associated with lower rates of diarrhea in pediatric populations (27). Interestingly, the same studies correlate the presence of Prevotella spp. with greater bacterial diversity. Prevotella spp. may be found in diets rich in complex polysaccharides and starchy fiber; our planned studies will therefore ascertain the presence or absence of these dietary components (28). In addition to these findings, the low abundance of Bifidobacterium in the patients without diarrhea suggests a preexisting dysbiosis as bifidobacteria are important for maintaining microbial homeostasis and can reduce levels of LPS that may be involved in the inflammatory component associated with VEGF-TKI induced diarrhea (29). This further suggests that mRCC patients taking VEGF-TKIs have an altered microbiota, which may relate to vulnerability to diarrhea.

As this was a pilot study aimed in part at determining the feasibility of stool bacteriomic profiling in this population, several caveats must be noted. Admittedly, our sample size was small. With a total sample of 20 patients, correction for false discovery was not possible. The sample was also heterogeneous in a number of respects. Patients receiving any VEGF-TKI were allowed to participate. Sunitinib, pazopanib, sorafenib, and axitinib, all have a varying affinity and specificity for the VEGF receptor. It is unclear whether off-target effects on platelet-derived growth factor receptor (PDGFR), Raf kinase, and other moieties could contribute disproportionately to gastrointestinal toxicity. Our study also did not control for dietary factors—thus, if patients were supplementing their diet with probiotics or ingesting foods fortified with bacteria (e.g., yogurt), this could have affected their stool bacteriomic profile. As no baseline stool sample was collected, it was unclear whether the VEGF-TKI had modified the bacterial flora of an individual patient, or if the stool bacterial composition was preexisting. Furthermore, in selected patients, samples were obtained during episodes of diarrhea. This limitation also prevents any conclusion related to the causative role the microbiota might have with regard to causing or preventing diarrhea; our results may instead reflect a change subsequent to incidence of diarrhea. Finally, the timing of stool collections was not uniform. Patients had received at least 2 weeks of VEGF-TKI therapy, by which time diarrhea has typically developed. Occasionally, however, these and other toxicities can occur in a latent fashion.

These caveats will be remedied in a follow-up study that is currently in development. In this validation study, a larger cohort of patients will be examined. These patients will be uniformly treated with a single VEGF-TKI. Stool will be collected at predefined timepoints, at baseline, after 2 weeks of therapy and after 3 months of treatment. An additional stool collection will be performed at the time of onset of diarrhea, and the CTCAE grade of diarrhea will be captured longitudinally (in contrast with the current study, a specific CTCAE grade will be assigned). Patients will be prohibited from changing their probiotic intake before the onset of diarrhea, along with bacteria-fortified foods while on therapy, and accurate dietary logs will be maintained. Furthermore, in a prospective fashion, a medication log will be maintained to ascertain whether patients could be taking agents that could prevent diarrhea (e.g., antimotility agents) or cause diarrhea (e.g., antibiotics). A separate cohort of healthy volunteers will also be accrued to validate the difference in bifidobacteria observed in the mRCC patients in the present study. In addition, several patients will have stool collected in repetition to confirm the reproducibility of our assays.

If this study confirms our preliminary findings of potentially protective bacterial species in the gastrointestinal flora, the next logical step will be an interventional study using a specially constructed probiotic supplement to mitigate VEGF-TKI–induced diarrhea. Through this stepwise process, it is hoped that an evidence-based approach to managing VEGF-TKI–induced diarrhea will emerge.

Disclosure of Potential Conflicts of Interest

S.K. Pal is a consultant/advisory board member for GlaxoSmithKline, Novartis, and Pfizer. C. Carmichael reports receiving speakers bureau honoraria from Novartis and Pfizer, and is a consultant/advisory board member for Bayer and Janssen. No potential conflicts of interest were disclosed by the other authors.

Authors’ Contributions

Conception and design: S.K. Pal, P. Frankel
Development of methodology: S.K. Pal, S.M. Li, X. Wu, H. Qin, P. Frankel
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): S.K. Pal, S.M. Li, X. Wu, J. Hsu, C. Carmichael

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Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): S.K. Pal, S.M. Li, X. Wu, M. Kortylewski, C. Carmichael, P. Frankel

Writing, review, and/or revision of the manuscript: S.K. Pal, S.M. Li, X. Wu, J. Hsu, C. Carmichael, P. Frankel

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): S.K. Pal, J. Hsu

Study supervision: S.K. Pal, S.M. Li

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