Tumor Genomic Profiling Guides Patients with Metastatic Gastric Cancer to Targeted Treatment: The VIKTORY Umbrella Trial

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The VIKTORY (targeted agent eValuation In gastric cancer basket KOREa) trial was designed to classify patients with metastatic gastric cancer based on clinical sequencing and focused on eight different biomarker groups (RAS aberration, TP53 mutation, PIK3CA mutation/amplification, MET amplification, MET overexpression, all negative, TSC2 deficient, or RICTOR amplification) to assign patients to one of the 10 associated clinical trials in second-line (2L) treatment. Capivasertib (AKT inhibitor), savolitinib (MET inhibitor), selumetinib (MEK inhibitor), adavosertib (WEE1 inhibitor), and vistusertib (TORC inhibitor) were tested with or without chemotherapy. Seven hundred seventy-two patients with gastric cancer were enrolled, and sequencing was successfully achieved in 715 patients (92.6%). When molecular screening was linked to seamless immediate access to parallel matched trials, 14.7% of patients received biomarker-assigned drug treatment. The biomarker-assigned treatment cohort had encouraging response rates and survival when compared with conventional 2L chemotherapy. Circulating tumor (ctDNA) analysis demonstrated good correlation between high MET copy number by ctDNA and response to savolitinib.

SIGNIFICANCE: Prospective clinical sequencing revealed that baseline heterogeneity between tumor samples from different patients affected response to biomarker-selected therapies. VIKTORY is the first and largest platform study in gastric cancer and supports both the feasibility of tumor profiling and its clinical utility.

INTRODUCTION

Recent advances in molecular analysis have revealed that there are patient subsets with differing genomic alterations despite the same histologic diagnosis in gastric cancer (1–3).

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design was based on the preclinical evidence of known molecular alterations, the prevalence of molecular alterations, and the availability of the targeted agents for clinical trials from AstraZeneca at the time of the study design. The candidate molecular alterations for the umbrella trial at the time of clinical trial design were molecular alterations in the p53, PIK3CA, MET, EGFR, FGFR2, RAS, and DDR pathways (3). Adavosertib is one of the most potent inhibitors targeting WEE1 (13), which is a tyrosine kinase that phosphorylates cyclin-dependent kinase 1 (CDK1, CD2C2) to inactivate the CD2C2/cyclin B complex (14). Inhibition of WEE1 activity prevents the phosphorylation of CD2C2 and impairs the G2 DNA-damage checkpoint, leading to cancer-cell death. Preclinical studies have demonstrated a very promising antitumor efficacy in vivo, especially in combination with other cytotoxic chemotherapeutic agents (15) including paclitaxel (16). Capivasertib is a selective pan-AKT inhibitor that inhibits the kinase activity of all three AKT isoforms (AKT1–3; ref. 17). Preclinically, sensitivity to capivasertib has been strongly correlated with the presence of PIK3CA mutations in gastric cancer models (18, 19). Savolitinib is a potent small-molecule reversible MET kinase inhibitor that inhibits MET kinase at an IC$_{50}$ of 4 nmol/L in MET-amplified cancer cells and has been shown to demonstrate promising antitumor activity in patients with gastric cancer (20, 21). Selumetinib (AZD6244, ARRY-142886) is a potent, orally active inhibitor of MEK1/2 that suppresses the pleiotropic output of the RAF/MEK/ERK pathway (22, 23). The tolerability and antitumor efficacy of the combination of selumetinib and docetaxel were demonstrated in patients with gastric cancer (24).

Herein we conducted a prospective clinical sequencing master program that was aligned with 8 prespecified genomic biomarkers and 10 independent biomarker-associated clinical trials in patients with metastatic gastric cancer. We explored whether the biomarker-selected platform trial benefits patients with metastatic gastric cancer in terms of survival. In addition, we investigated PD-L1 score and circulating tumor (ctDNA) change between baseline and post-treatment samples following targeted agents.

RESULTS

Patient Characteristics

Between March 2014 and July 2018, 772 patients with metastatic gastric cancer were enrolled onto the VIKTORY trial. Targeted sequencing was successfully achieved with tissues from 715 patients (92.6%; Fig. 1A and B). Of the 715 tissues, 150 (21.1%) were from fresh tumors, 564 (78.9%) from formalin-fixed paraffin-embedded (FFPE) specimens, and 1 from ctDNA sequencing using Guardant360 (Fig. 2A). Nearly all samples (96.2%) were from the primary gastric tumor specimen; 56.4% of the patients had their tumor at the time of diagnosis of metastatic gastric cancer and 43.6% of patients were sequenced during first-line (1L) or at the time of progression following 1L chemotherapy. The tissue type, site of biopsy for sequencing, and Epstein–Barr virus (EBV) and mismatch-repair (MMR) status of the 715 patients are summarized in Fig. 2A. A total of 75.9% of patients had poorly differentiated adenocarcinoma.

The primary tumor was located in the body (53.2%) or antrum (37.7%) of the stomach in the majority of patients. All patients underwent 1L cytotoxic chemotherapy (>85% with fluoropyrimidine/platinum regimen). In all, 460 of 715 patients (64.3%) were eligible for 2L therapies: 143 of 715 (20.6%) were assigned to one of the umbrella-associated parallel clinical trials in 2L (105 with Biomarker A–E or G; 38 with Biomarker F, unscored), whereas 317 patients received conventional treatment or treatment via other clinical trials (Figs. 1B and 2A).

Tumor Genome Profiling

The tumor profiles of the 715 patients are shown in Supplementary Fig. S1, and the detailed sequencing method is provided in the Supplementary Material. The prevalence of the predefined biomarkers was as follows (Fig. 2B): Biomarker A1: RAS mutation/amplification (81/715, 12.2%; KRAS 62/715, 8.7%; HRAS 6/715, 0.8%; NRAS 19/715, 2.7%); Biomarker A2: high or low MEK signature (49/107, 45.8%); Biomarker B: TP53 mutation (321/715, 44.9%); Biomarker C: PIK3CA mutation/amplification (54/715, 7.6%); Biomarker D: MET amplification (25/715, 3.5%); Biomarker E: MET overexpression by IHC 3+ (42/479, 8.8%); Biomarker F: none of the above (Biomarker A–E); Biomarker G: RICTOR amplification (5/715, 0.7%)/TSC2 deficient (7/715, 0.9%). In addition to the predefined biomarkers, we identified other known molecular targets in gastric cancer (Supplementary Fig. S1): FGFR2 amplification (30/715, 4.2%), EGFR amplification (17/715, 2.4%), MDM2 amplification (8/715, 1.1%), AKTI amplification (2/715, 0.3%), FGFR1 amplification (10/715, 1.4%), and CCNE1 amplification (14/715, 2.0%). In all, 3.5% were MMR-deficient gastric cancer (18/523) and 4% (20/501) were EBV-positive. Concurrent MMR and EBV status are provided in 105 patients treated according to biomarker status (Fig. 2B, left). In addition, concurrent molecular profiling of each patient according to biomarker (e.g., KRAS mutation and TP53 mutation) and the assigned umbrella arm is summarized in Fig. 2B (right) according to the biomarker priority. The incidence of MET overexpression by IHC (defined by 3+) was 8.8% (42/479) in this cohort: 17 (40.5%) of 42 MET-overexpressed tumors had MET-amplified tumors by next-generation sequencing (NGS) or FISH, and 25 (59.5%) patients had no MET amplification, which concurred with our previous finding on coactivation of MET protein without amplification (25, 26).

Treatment Efficacy of the Umbrella Trial

The cutoff date for treatment outcome analysis was October 1, 2018. At the time of analysis, enrollment had been completed in all arms or stopped due to early termination of drug development (arms 6, 9, 10) or lack of efficacy at first stage of phase II arm 7; Supplementary Table S1). Currently, enrollment is completed in phase I of arm 8, and phase II is being considered. Further patient enrollment was halted in arm 5 (savolitinib/docetaxel combination) due to the high efficacy observed with the savolitinib monotherapy arm. The primary endpoint was overall response rate (ORR); assuming ORR of 20% for 2L paclitaxel, experimental arms were considered effective if the combination yielded ≥50% ORR for arms 1–10 except for arm 4 (savolitinib...
A

Patients with metastatic GC

Enrolled for VIKTORY screening
56.4% at the time of 1st-line chemotherapy
43.6% during or at the time of failure to 1st-line chemotherapy

Tumor pathologic–genomic profiling:
1) Targeted tumor sequencing
2) NanoString (MEK signature)
3) IHC panel: MMR, EBV status, PD-L1, c-MET
4) Serial ctDNA sequencing

B

n = 772, Patients with GC were consented to VIKTORY umbrella screening program

n = 57, Excluded
n = 48, QC failed for targeted sequencing
n = 9, Other reasons (e.g., consent withdrawal)

n = 715, tumor specimens passed QC/sequenced

n = 255, Not eligible for 2nd-line treatment
n = 157, Poor PS or followup loss
n = 48, Not progressed on 1st-line Tx

n = 460, Patients eligible for 2nd-line treatment

n = 143, Assigned to umbrella associated arms

n = 317, Non–umbrella treatment
n = 99, Taxol/ramucirumab
n = 105, Taxane-based chemotherapy
n = 62, Irinotecan-based chemotherapy
n = 27, Biomarker-specific sponsored trials (non-VIKTORY)

n = 24, Immunotherapy trials

Planned biomarker-negative trial (n = 38)
1) Planned biomarker-negative PI (biomarker exploratory) (n = 27)
   (vistusertib + paclitaxel (n = 16), capivasertib + paclitaxel (n = 11))
2) Biomarker-negative PI trials (dose finding)
   (AZD6738 + paclitaxel (n = 9; PI), savolitinib + docetaxel (n = 2; P2))

n = 105, Assigned to biomarker-specific trials

Arm 1: Selumetinib + docetaxel
   KRAS mt or amp
Arm 2: Adavosertib + paclitaxel
   TPS3 mutation
Arm 3: Capivasertib + paclitaxel
   PIK3CA mt or amp (n = 24)
Arm 4: Savolitinib
   MET amp (n = 25)
Arm 4-1: Savolitinib + docetaxel
Arm 5: Savolitinib + docetaxel
   MET 3+ by IHC (n = 4)
Arm 6: Vistusertib + paclitaxel
   TSC2 null
Arm 7: Vistusertib + paclitaxel
   RICTOR amp
Arm 8: Vistusertib + paclitaxel
   TSC2 null
   RICTOR amp
Arm 9: Vistusertib + paclitaxel
   TSC2 null
   RICTOR amp
Arm 10: Vistusertib + paclitaxel
   TSC2 null
   RICTOR amp

Figure 1. An overview of the VIKTORY trial design. A, The study design of the VIKTORY trial. B, The patient allocation schema of the trial. PII, phase II; PI, phase I; GC, gastric cancer; QC, quality control; PS, performance status; mt, mutation; amp, amplification.
A

VIKTORY umbrella trial \((n = 715)\)

- Targeted seq: Yes (715, 100%)
- FF vs. FFPE: N/A (n = 214)
- Site of biopsy for sequencing: N/A (n = 12)
- EBV status: Positive (210/523, 40.2%)
- Negative (315/523, 60.0%)
- N/A (n = 2)
- MMR status: p-MMR (505/523, 96.5%)
- d-MMR (18/523, 3.5%)
- N/A (n = 2)
- Assigned umbrella arm:
  - Arm 1: PIK3CA mutation: Selumetinib/docetaxel (25, 3.5%)
  - Arm 2: RAS mt/amplification: Adavosertib/paclitaxel (25, 3.5%)
  - Arm 3: PIK3CA mt/ampl: Capivasertib/paclitaxel (4, 0.6%)
  - Arm 4: MET amp: Savolitinib (20, 2.8%)
  - Arm 4-1: MET amp: Savolitinib/docetaxel (4, 0.6%)
  - Arm 5: MET overexp: Savolitinib/docetaxel (4, 0.6%)
  - Arm 6: PIK3CA mutation: Vistusertib/docetaxel (4, 0.06%)
  - Arm 7: RAS mt/amplification: Vistusertib/docetaxel (4, 0.6%)
  - Arm 8: MET overexp: Savolitinib/docetaxel (4, 0.6%)
  - Arm 9: RICTOR amp: Vistusertib/paclitaxel (4, 0.6%)
  - Arm 10: RAS mt/amplification: Vistusertib/paclitaxel (4, 0.6%)

B

Assigned umbrella arm

- Arm 1: PIK3CA mutation (n = 25)
- RAS mt/amplification (n = 22)
- MET amplification (n = 23)
- TP53 mutation (n = 48)
- MET overexpression (n = 21)
- PIK3CA mutation (n = 25)
- RAS mt/amplification (n = 22)
- MET amplification (n = 23)
- TP53 mutation (n = 48)
- MET overexpression (n = 21)
- PIK3CA mutation (n = 25)
- RAS mt/amplification (n = 22)
- MET amplification (n = 23)
- TP53 mutation (n = 48)
- MET overexpression (n = 21)
monotherapy arm). The ORR for each umbrella arm was as follows: arm 1 (selumetinib/docetaxel): 28% (7/25, 95% CI: 10.4–45.6), arm 2 (adavosertib/paclitaxel): 24% (6/25, 95% CI: 7.3–40.7), arm 3 (capivasertib/paclitaxel): 33.3% (8/24, 95% CI: 14.4–52.2), and arm 4 (savolitinib): 50% (10/20, 95% CI: 28.0–71.9; Supplementary Table S1 for detailed primary endpoints for each arm). The waterfall plots and swimmer plots are provided in Fig. 3. Seven of 25 patients who had a partial response (PR) in the selumetinib/docetaxel arm (arm 1) had KRAS amplification/high MEK (MEK-H), KRAS wild-type (WT)/low MEK (MEK-L), KRAS G12R/MEK-H, KRAS G12D/MEK-L, KRAS G12D/MEK-H, KRAS G13D/MEK-I, KRAS WT MEK-H, and KRAS Q61R/MEK-I, respectively. The longest responder carried a KRAS amp (KRAS WT) with high MEK signature (arm 1-005; Fig. 3A, top right). In terms of KRAS mutational status, there was no significant difference in ORR between KRAS-mutant (4 of 11, 36.4%) and KRAS WT (3/14, 21.4%; P = 0.538, χ² test). For the Biomarker B–arm 2 (adavosertib/paclitaxel) umbrella, there were six PRs (6/25) and 3 of these patients responded longer than 6 months (Fig. 3B). For Biomarker C–arm 3 (capivasertib/paclitaxel), there were 8 responders (8/24) with 4 patients responding for more than 6 months (Fig. 3C). For Biomarker D–arm 4 (savolitinib monotherapy), there were 10 PRs (10 of 20) of whom (arm 4-010) had the tumor resected after achieving complete response (CR; Fig. 3D). This patient was a 65-year-old female who was laparoscopically diagnosed with peritoneal seeding at diagnosis. After failing the 1L capcitabine/oxaliplatin treatment and developing rapidly deteriorating malignant ascites, the patient was assigned to savolitinib due to high copy-number MET amplification. After significant tumor reduction following savolitinib, the patient underwent curative resection and achieved pathologic downstaging from M1 disease to T3N2M0 disease. The patient remains in CR, now more than 1 year at the time of manuscript preparation.

**Prediction of Best Clinical Response Based on Genomic Variations for Individual Patients with Gastric Cancer**

Genomic variations are increasingly being utilized as reliable biomarkers for predicting clinical response to therapy for gastric cancer (27–29). To identify genomic variants that significantly correlate with clinical response, we compared the maximal tumor burden change per RECIST 1.1 against single genomic alterations (Fig. 4A). Patients with MET amplifications demonstrated the largest absolute decrease in tumor burden per RECIST 1.1. In addition, PIK3CA helical domain E542K patients had a more profound (≥50%) reduction in tumor burden when compared with patients with other point mutations in PIK3CA–E545G, E545K, E545K, H1047R, C420R, or E453K. Among patients with TP53 mutations, R273C, R175H, R342X, and Y220C demonstrated the most tumor reduction upon adavosertib/paclitaxel therapy. Finally, patients with KRAS G13E and KRAS G12D mutations, KRAS amplification, or MEK-H without KRAS mutation demonstrated the highest tumor burden reduction by selumetinib/docetaxel. Further focused genomic analysis of the Biomarker D (MET amplification) group and treatment response to savolitinib demonstrated that patients with gastric cancer with high MET copy number (>10 MET gene copies by tissue NGS) had high response rates to savolitinib (Fig. 4B). Patient arm 4-010 who initially had gastric cancer with peritoneal seeding had a MET tissue NGS copy number of 25.9 and achieved PR following savolitinib, which eventually led to curative surgery, as mentioned previously. Although limited by the small number of patients, 5 responders to savolitinib had PD-L1–positive tumors [range, 3 to 80 for combined positive score (CPS)]; including patient arm 4-010 (Fig. 4B). Another focused genomic analysis of the Biomarker C (PIK3CA mutation) group and treatment response to capivasertib/paclitaxel showed that 57.1% (4 of 7 PRs) had E542K mutations. Moreover, patients with PIK3CA E542K mutations demonstrated an ORR of 50% (4/8), which was higher than the non-E542K cohort (3/16, 18.8%; P = 0.063 by χ²; Fig. 4C). Toxicity profiles for the four arms are shown in Supplementary Table S2.

**Survival Analysis**

We conducted an OS analysis on the biomarker-driven treatment group using the Kaplan–Meier plot in all patients. In all, patients with gastric cancer who had biomarkers identified and were treated accordingly (N = 105) demonstrated better OS (median OS, 9.8 months) when compared with patients who received conventional 2L therapy (N = 266; taxol/ramucirumab, N = 99; taxane-based, N = 105; irinotecan-based, N = 62) treatment (median OS, 6.9 months) with statistical significance (P < 0.001; Fig. 5A). The results from the biomarker-driven treatment cohort retained statistical significance in a multivariate analysis, and this treatment continued to predict better survival (P < 0.0001, HR = 0.58; 95% CI: 0.45–0.76) after correcting for potential prognostic factors such as age, gender, number of involved organs, EBV status, MMR status, and performance status (Fig. 5B). Concordantly, the VIKTORY biomarker-assigned cohort (N = 105) had significantly prolonged progression-free survival (PFS) when compared with the conventional 2L cohort (N = 266; median PFS, 5.7 months vs. 3.8 months, respectively, P < 0.0001; Fig. 5C). The multivariate Cox regression analysis for PFS revealed that being biomarker positive was an independent prognostic factor after adjustment for the several clinically important factors (Supplementary Fig. S2). Hence, when the biomarker was identified and the patient received a matched treatment with targeted agents at an appropriate time, patients had prolonged PFS and OS compared with conventional chemotherapy.
Figure 3. The drug efficacy data. The left panel shows the waterfall plot and the right panel demonstrates the swimmer plot. The y-axis represents % of maximum tumor reduction assessed according to RECIST 1.1 criteria. 

A, Arm 1: selumetinib (MEK inhibitor)/docetaxel arm for patients with RAS-aberrant gastric cancer. 
B, Arm 2: adovasertib (WEE1 inhibitor)/paclitaxel arm for patients with TP53-mutant gastric cancer. 
C, Arm 3: capivasertib (AKT inhibitor)/paclitaxel arm for patients with PIK3CA-mutant gastric cancer. 
D, Arm 4: savolitinib (MET inhibitor) monotherapy arm for patients with MET-amplified gastric cancer. * indicates newly developed lesion per RECIST 1.1. SD, stable disease.
Figure 4. Molecular alterations and drug efficacy for each patient. A, Demonstration of each patient's tumor profile and the maximal tumor size with each drug. (continued on next page)
Figure 4. (Continued) B, Molecular landscape of the patients enrolled in savolitinib monotherapy (arm 4). C, Molecular landscape of the patients enrolled in capivasertib/paclitaxel (arm 3). Heat map showing the mutational landscape of patients. Bar plot showing mutation counts. R, responder; NR, nonresponder; N/A, not available.
Figure 5. Survival outcome. Survival analysis of the patients with gastric cancer who were treated according to biomarker as 2L treatment (N = 105) versus conventional 2L treatment (N = 266; taxol/ramucirumab, N = 99; taxane-based, N = 105; irinotecan-based, N = 62) A, OS subgroup analysis for 371 patients with gastric cancer who underwent any 2L treatment; B, HRs for OS; C, PFS subgroup analysis for 371 patients with gastric cancer who underwent any 2L treatment.
Changes in ctDNA and PD-L1 Expression after Treatment

On the basis of the tumor heterogeneity and genomic changes we observed in our previous studies (11, 27, 30), we collected plasma for ctDNA analysis at baseline and every CT evaluation until progression to address tumor evolution. The concordance rate between tumor and ctDNA (tested by Guardant360; Supplementary Table S3) for MET amplification was 89.5%, with 100% specificity and 83.3% sensitivity relative to tissue testing, which increased to 100% if patients without detectable ctDNA were excluded (Fig. 6A). The maximal tumor burden decrease was observed in patients with high adjusted MET copy number by ctDNA, although statistical significance was not reached (Fig. 6B). More importantly, however, increased adjusted plasma copy number for MET amplification was significantly associated with prolonged PFS on savolitinib (Fig. 6C; \( P = 0.0216 \)) to a significantly greater degree than tissue NGS MET copy number, which may reflect plasma’s ability to synthesize the entire tumor cell population. Savolitinib therapy markedly decreased total ctDNA levels in all patients for which baseline and 4-week plasma results were available (Fig. 6D), demonstrating clear biological activity before most radiographic evidence of response. Congruently, adjusted plasma MET copy number was markedly suppressed at 4 weeks in all patients for whom results were available, although 2 of the 6 patients tested retained detectable MET amplification on progression, suggesting additional off-target mechanisms of acquired resistance (Fig. 6E).

We additionally sequenced 55 (from 29 patients) ctDNA samples from arm 1 (13 patients) and arm 2 (16 patients) using a 300-gene AstraZeneca (AZ) panel (Supplementary Table S4 and S5; Fig. 6F and G). Concordance between tumor DNA and ctDNA was observed in 10 of 13 (76.9%) patients for KRAS aberration status (arm 1) and 75% (12 of 16) for TP53 mutation status (arm 2; Fig. 6F and G). Of the 8 baseline/progressive disease (PD) paired ctDNA samples in arm 1, only 2 (25%) had retained baseline genomic alterations at disease progression. Of the 11 baseline/PD paired ctDNA samples in arm 2, 5 (45.5%) patients showed no major alterations at disease progression in the 300-gene panel following adavosertib/paclitaxel treatment. Dynamic changes from baseline to disease progression in ctDNA mutational count using the AZ 300-gene panel are shown in Supplementary Fig. S3.

Finally, we analyzed PD-L1 score in 230 patients, which revealed that 30.4% (70 of 230) had PD-L1 CPS ≥ 1. In this subset, we had 25 paired biopsy specimens (baseline and at PD to one of the VIKTORY regimens) available for PD-L1 analysis (Supplementary Table S6). All baseline and post-treatment biopsies were obtained from the same primary stomach lesion. Of the 25 paired samples analyzed, there were 2 patients (both treated with selumetinib/docetaxel) who showed a significant increase in PD-L1 (CPS ≥ 10) at progression after 5 to 8 months of selumetinib/docetaxel treatment (Fig. 7A). Arm1-019 patient developed multiple somatic mutations at the time of progression on selumetinib/docetaxel treatment by ctDNA analysis (Fig. 7B).

DISCUSSION

To our knowledge, this is the first and largest study to use an umbrella platform trial design with preplanned genomic biomarker analyses to assign patients with advanced gastric cancer to molecularly matched therapies. Using a centrally standardized molecular screening protocol, we enrolled 772 patients with gastric cancer and successfully performed tissue analysis for more than 90% (92.6%) of the patients as reported in our previous studies (28, 31). In this study, we demonstrated that when comprehensive molecular screening is linked to seamless immediate access to parallel matched trials, nearly 1 in 7 (14.7%) patients with advanced gastric cancer can receive biomarker-assigned drug treatment. The proportion of biomarker-driven treatment (14.7%) can be increased if the availability of seamless parallel trials is increased (i.e., FGFR2 amplification, EGFR amplification). Importantly, we showed that the biomarker-assigned cohort had encouraging response rates, underscoring the importance of genomically characterizing every patient’s tumor for precision therapy.

Of the multiple arms, the highest response rate was observed in arm 4 (MET amplification–savolitinib monotherapy). Savolitinib is a potent small-molecule reversible MET kinase inhibitor that inhibits MET kinase with an IC\(_{50}\) of 4 nmol/L in MET-amplified cancer cell lines. A phase II trial of savolitinib monotherapy in 44 patients with MET-altered papillary renal cell carcinoma showed very promising results, including 8 PRs (32). Our savolitinib monotherapy arm met the prespecified 6-week PFS rate, indicating that this treatment is worthy of phase III exploration in the MET-amplified subset of patients with gastric cancer (3%–5%; refs. 33, 34). Responders were enriched for higher MET copy number (7/10 with MET >10 copies), a biological phenomenon reported in our previous studies (28, 31). In this study, we observed in arm 4, (MET amplification–savolitinib monotherapy) series.

Amplification of genomic biomarker context, concurrent receptor tyrosine kinase (RTK) amplifications in addition to MET amplification resulted in short duration of response or no response to savolitinib. The importance of understanding the concurrent alteration landscape is highlighted by mixed results with prior MET-directed therapies in gastric cancer, likely owing to incomplete biomarker selection (37–39). Although this analysis is lacking functional validation, we speculate tumors with higher MET copy number without other RTK coamplifications are more dependent on MET signaling and may represent the optimal candidates for MET-directed therapy.
The VIKTORY Umbrella Trial in Gastric Cancer

A

Tissue
+  10  0  PPA  83.3%
-  0  5  NPA  100%
Plasma
+  2  2  PPV  100%
-  2  2  Concordance  89.5%

B

Greatest change in lesion size (%)

R²  P
-  0.0012  0.9294
-  0.1165  0.3687
-  0.03203  0.701
-  0.01464  0.425

C

PFS (weeks)

R²  P
-  0.01464  0.7392
-  0.5031  0.0216
-  0.1088  0.425
-  0.01464  0.7392

D

ctDNA amplification change (proportion initial)

E

cDNA MET amplification (proportion adjusted copy number)

F

Selumetinib arm: paired baseline/PD cfDNA

G

Adavosertib arm: paired baseline/PD cfDNA

Key to alterations
Amplification
Splice site mutation
Truncating mutation
Deletion
Missense mutation
Frameshift mutation

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Figure 7. Changes in PD-L1 after docetaxel/selumetinib treatment. A, Changes in PD-L1 score between baseline and at disease progression following 8 months of selumetinib/docetaxel treatment from patient arm1-019. IHC for T-cell markers (CD3 and CD8) showed a dramatic increase in T cells after treatment (left two columns). Likewise, patient arm1-012 demonstrated a dramatic increase in PD-L1 CPS that was accompanied by increase in CD3+ and CD8+ lymphocyte infiltration following 5 months of selumetinib/docetaxel treatment. All biopsies were obtained from primary stomach cancer tissue. B, For patient arm1-019, the genomic landscape of ctDNA changed during selumetinib/docetaxel treatment with newly emerged mutations at disease progression. VAF, variant allele frequency.
therapies. Of note, patients with gastric cancer with a high level of ctDNA MET amplification (by Guardant360 assay in our study) may benefit more substantially from MET-targeted therapy.

In arm 3 (PIK3CA mutation—capivastib), we observed moderate antitumor activity with an ORR of 33.3% (95% CI: 14.4–52.2) in 2L gastric cancer, especially when compared with the low response rate (<1%) observed in arm 7 (PIK3CA WT—capivastib). Capivastib is a selective pan-AKT inhibitor that inhibits the kinase activity of all three AKT isoforms (AKT1–3; ref. 17). We and others have previously observed differential distribution of PIK3CA hotspot mutations (E542K, E545K, H1047R) according to molecular subtypes—PIK3CA kinase domain H1047R mutations were enriched in microsatellite instability–high (MSI-H) gastric cancer (>80%), whereas helical domain E542K and E545K mutations were enriched in microsatellite-stable (MSS) tumors (1, 40). Given that each molecular subtype (MSI-H, MSS, genomically stable, or mesenchymal subtype) has substantially different survival outcomes (1), we have hypothesized that specific point mutations may show different drug sensitivity to capivastib. Among arm 3 patients, we observed strikingly different efficacy based on PIK3CA genotype (Fig. 4C). In fact, none of the 4 patients with H1047R PIK3CA mutations responded to capivastib. In contrast, 4 of the 8 with E542K mutations had durable responses to the capivastib/docetaxel combination, and 3 of the 4 patients were EBV-positive (Fig. 3C, green circles). Taken together, capivastib/docetaxel demonstrated the highest antitumor activity in MSS gastric cancer with PIK3CA E542K mutations. While this represents the first trial of a pan-AKT inhibitor in PIK3CA-mutated gastric cancer, randomized data will be important to validate our putative composite biomarker (PIK3CA helical domain +MSS) population.

MAPK pathway alterations are frequent in advanced gastric cancer. We attempted to explore two biomarker selection strategies using selumetinib (AZD6244, ARRY-142886), which is a potent, orally active inhibitor of MEK1/2 that suppresses the pleiotropic output of the RAF/MEK/ERK pathway (22, 23). First, we confirmed that KRAS mutational status did not predict response to selumetinib in patients with gastric cancer, supporting the preclinical data with MEK inhibitors (23). On the basis of a study showing that the Ras pathway can be activated in the absence of KRAS mutation and that the Ras-pathway signature was superior to KRAS mutation status for the prediction of response to Ras-pathway inhibitors (41), a 6-gene MEK signature (DUSP4, DUSP6, ETV4, ETV5, PHLD1A, and SPRY2) was developed and validated in the gastric cancer cohort (42). Given that the prevalence of high MEK signature was only 6.9%, the predictive power of high MEK signature should be tested in a subsequent enriched clinical trial with high MEK signature as a selection biomarker in gastric cancer. Interestingly, we observed the most durable response in a KRAS amplification/MEK-H patient without concurrent KRAS mutation, consistent with recent reports of MEK inhibition in this genomically defined subset (43).

Recent trials have underscored the importance of anti-PD-1 or anti–PD-L1 therapy in gastric cancer treatment especially in patients with metastatic gastric cancer with EBV-positive or high mutational load or MSI-H or PD-L1 combined CPS ≥ 1 by IHC (27, 44). We observed substantial induction (increase of ≥10) of PD-L1 in 8% (2/25) of paired biopsies from primary tumors in the selumetinib/docetaxel arm (Supplementary Table S6). MAPK inhibition by cobimetinib in preclinical tumor models has been shown to promote tumor-infiltrating CD8+ T-cell activity (45). In addition, atezolizumab and cobimetinib combination treatment has been shown to increase intratumoral CD8+ T-cell infiltration and MHC I expression in patients with MSS colorectal cancer (46). Concordantly, we also observed PD-L1 change with recruitment of intratumoral CD8+ lymphocytes following selumetinib/docetaxel treatment. Although a recent cobimetinib/atezolizumab trial has failed to show survival benefit in patients with MSS colorectal cancer (47), selumetinib and anti–PD-L1 treatment may be explored in patients with MSS gastric cancer. Congruently, this highlights the nonstatic nature of PD-L1 as a selection biomarker and suggests combination and/or sequential strategies are worth exploration.

Although we are a long way from claiming “VIKTORY” in gastric cancer, we have successfully shown that tumor genomic profiling with matched therapies improves outcomes in 2L treatment, and that platform clinical trials can efficiently identify the optimal biomarker treatment match (e.g., savolitinib to patients with MET-amplified gastric cancer). Nevertheless, this signal needs to be confirmed in an expansion or randomized trial. Exploratory analyses demonstrated that biomarkers such as genomic alterations and/or PD-L1 may not be static, especially during or after treatment. The proportion (14.7%) of patients receiving biomarker-driven treatment in the VIKTORY trial may be improved with more available targeted agents based on genomic alterations (e.g., FGFR2 or EGFR2 amplification) and inclusion of PD-L1 positivity (especially PD-L1 CPS ≥10) may enable interrogation of the potential benefit of anti–PD-L1 treatment with or without targeted agents in future umbrella trials. Finally, although limited by a very small number of patients, we have demonstrated that PD-L1 status changes over time in gastric cancer following selumetinib/docetaxel treatment.

METHODS

Patient Selection

Patients with histologically confirmed metastatic and/or recurrent gastric adenocarcinoma, an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1, and at least one measurable lesion according to the RECIST 1.1 were eligible for enrollment in the VIKTORY trial, the molecular screening program, and one of the associated umbrella trial protocols in gastric cancer. Adequate hematologic function, hepatic function, and renal function were required. Patients with other concurrent uncontrolled medical diseases and/or other tumors were also excluded. The trial was conducted in accordance with the Declaration of Helsinki and the Guidelines for Good Clinical Practice (ClinicalTrials.gov identifier: NCT02299648). The trial protocol was approved by the institutional review board of Samsung Medical Center (Seoul, Korea) and all participating centers, and all patients provided written informed consent before enrollment.
Study Design

The main goal of the VIKTORY trial as a molecular screening program was to identify novel molecular subsets for assigning patients into one of the associated biomarker-directed arms (Fig. 1A). There were 10 associated independently operated phase II arms (arms 4 and 8 included a dose-finding phase I trial) with eight biomarkers. Each experimental drug protocol was designed independently from the screening protocol. The eight biomarkers were: Biomarker A1: RAS mutation or RAS amplification; Biomarker A2: high MEK (MEK-H) or low MEK (MEK-L) signature; Biomarker B: TPS3 mutation; Biomarker C: PIK3CA mutation or amplification; Biomarker D: MET mutation; Biomarker E: MET overexpression (3+) without MET amplification; Biomarker F: all negative (TPS3 WT/PIK3CA WT/RAS WT), and Biomarker G: TSC2 null or RICTOR amplification. There were 10 phase II trials that were associated with the VIKTORY screening protocol: arm 1: selumetinib + docetaxel (Biomarker A1/A2, NCT#02448290); arm 2: adavosertib + paclitaxel (Biomarker B, NCT#02448329); arm 3: capivasertib + paclitaxel (Biomarker C, NCT#02451956); arm 4: savolitinib monotherapy (Biomarker D, NCT#02449551); arm 4: savolitinib + docetaxel (Biomarker D, NCT#02447406), arm 5: savolitinib + docetaxel (Biomarker E, NCT#02447380); arm 6: T/8: vistusertib + paclitaxel or capivasertib + paclitaxel (Biomarker F, NCT#02449655) or AZD6738 + paclitaxel (NCT#2630199); and arm 9–10: vistusertib + paclitaxel (Biomarker G, NCT#03082833, NCT#02449655), vistusertib + paclitaxel (Biomarker G, NCT#03061708). If patients initially enrolled in the VIKTORY trial were not eligible or refused to participate in one of the associated trials, they were allowed to be treated with conventional chemotherapy, or in non-VIKTORY clinical trials.

Sample Collection and IHC

FFPE or fresh samples of gastric cancer containing >40% tumor cellularity were used for targeted sequencing. Genomic DNA was extracted using the Qagen DNA kit for FFPE tissue or the QIAGen DNA Mini Kit for fresh tumor tissues (Qiagen) according to the manufacturer’s instructions. The IHC protocol for MET and HER2 used for this trial has been reported previously (48). The remaining tissue samples were reused in case of insufficient DNA amount/quality for molecular analysis, or otherwise stored for further study.

Tissue DNA Targeted Sequencing

The targeted sequencing method for tissue specimens is provided in the Supplementary Material.

PD-L1, CD3, and CD8 IHC

Tissue sections were freshly cut to 4 µm-thick sections and mounted on Fisherbrand Superfrost Plus Microscope Slides (Thermo Fisher Scientific) and then dried at 60°C for 1 hour. IHC staining was carried out on Dako Autostainer Link 48 system (Agilent Technologies) using Dako PD-L1 IHC 22C3 pharmDx kit (Agilent Technologies) with EnVision FLEX visualization system and counterstained with hematoxylin according to the manufacturer’s instructions. PD-L1 protein expression was determined using CPS, which was the number of PD-L1-staining cells (tumor cells, lymphocytes, macrophages) divided by the total number of viable tumor cells, multiplied by 100. The specimen was considered to have PD-L1 expression if CPS was ≥2%. For CD3 and CD8, IHC staining was performed on tissue sections from FFPE-embedded specimens with VENTANA BenchMark automated staining instrument (Ventana Medical Systems, Inc.). Specimens were incubated with CONFIRM anti-CD3 (2G6V) and CONFIRM anti-CD8 (SP57) rabbit mAbs for 20 minutes and CD3+ and CD8+ immune cells were visualized using the OptiView DAB IHC Detection Kit.

MMR Determination and EBV In Situ Hybridization

Antibodies used in this study were specific for MLH-1 (M1, Ventana, ready to use) using Ventana BenchMark XT autostainer (Ventana); MSH2 (G219-1129, 1,500, Cell Marque), PMS2 (MRQ-28, 1,20, Cell Marque), and MSH6 (44/MSH6, 1,500, BD Biosciences) using BOND-MAX autoimmunostainer (Leica Biosystems). In interpretation, loss of nuclear staining in the tumor cells with positively stained internal control was counted as an abnormal result. In cases with loss or suspected as loss of MMR protein, IHC was initially selected and further IHC with the entire block was performed to screen for MMR deficiency. Cases with negative or equivocal nuclear staining were subsequently tested for microsatellite instability using PCR. EBV status was determined by EBER in situ hybridization using standard protocols (27).

cDNA Purification

cDNA testing using Guardant360 (Guardant Health) was performed as described previously (49). Briefly, up to 30 ng of cell-free DNA extracted from banked plasma was used for library preparation and enrichment by hybridization capture. Enriched libraries were then sequenced on a NextSeq 550 (illumina), and the resulting sequence data was analyzed using a locked, previously validated custom bioinformatics pipeline. Plasma copy number was reported as directly observed and adjusted as described previously (50). Change in total ctDNA levels was calculated as described previously (51) and reported as proportional fold change truncated at 10% for graphical purposes.

Treatment Allocation Procedure

The molecular tumor board (MTB) was composed of medical oncologists, pathologists, bioinformaticians, and the small-molecule experts from AstraZeneca. The MTB had the responsibilities of scientific validation, prioritization of identified molecular aberrations, and providing guidance on the suitable biomarker-driven experimental arm under the umbrella trial. The process time between biopsy and molecular results was set as 21 to 30 days from our previous study (28, 31). If multiple targets were simultaneously detected in a single patient, the following prioritization was used for patient assignment based on known drivers: (i) PIK3CA mutation/amplification; (ii) RAS mutation/amplification or MEK signature; (iii) MET amplification; (iv) TP53 mutation; (v) RICTOR amplification; (vi) TSC2 null; (vii) MET overexpression by IHC 3+; and (viii) if none of the above biomarkers were present, patients were allocated to the biomarker-negative arms AZD6738/paclitaxel, capivasertib/paclitaxel, phase 1 portion of docetaxel/savolitinib, other clinical trials, or conventional treatment. The status of enrollment for 10 associated clinical trials (10 phase II studies) is shown in Supplementary Table S1. Patient enrollment has been completed in arms 1, 2, 3, 4, 5, 6, and 7. Further patient enrollment was stopped in arms 4–6 and 8, and arms 9/10 have been closed early due to early termination of the drug for further clinical development.

Statistical Analysis

This trial was designed as two parts: (i) VIKTORY screening protocol for molecular profiling; (ii) parallel phase I/II study with independent statistical assumptions for each arm. For each arm, the primary endpoint was ORR. We adopted Simon’s optimal design assuming ORR of 20% for 2L weekly paclitaxel regimen based on robust data from previous studies: the experimental arm (paclitaxel + targeted agents) was considered effective for further development if the combination rendered an ORR of ≥50%. Each arm was designed as a two-stage design, allowing ineffective drugs to be terminated early at stage 1. Secondary endpoints were PFS, OS, and correlative biomarker analysis using ctDNA, PD-L1 score, and genomic aberration.
Statistical analyses were performed using the software environment R v3.4.0. The clinical information distribution plots were created using Circos (52). Survival analyses were performed to explore the influences of age, gender, pathology, disease status, the number of metastatic organs, EBV status, MMR status, PD-L1 status, and VIKTORY biomarker status. Survival function curves were visualized using the library and the differences between the levels of each factor were assessed using a log-rank test. Likewise, to model hazard functions and determine the effects of these factors on a patient’s survival, Cox proportional hazard models were used. The proportional hazard assumption of Cox models was tested using the R library survival (53). The significance of multiple predictors of survival was assessed by the Cox regression analysis. P < 0.01 was considered to indicate a statistically significant difference. The forest plot of the HRs according to the OS was generated using in-house code. We used the lollipop chart to visualize the maximum change in tumor size per RECIST 1.1.

Disclosure of Potential Conflicts of Interest
I. Kozarewa has ownership interest (including stock, patents, etc.) in AstraZeneca plc and has ownership interest (including stock, patents, etc.) in the same. J.I. Odegaard is a VP, clinical development at Guardant Health. E.A. Harrington has ownership interest (including stock, patents, etc.) in AstraZeneca. S. Luke has ownership interest (including stock, patents, etc.) in AstraZeneca and Illumina. Y.H. Kim has ownership interest in AstraZeneca stock and is a consultant/advisory board member at Orum Therapeutics. A.A. Talasaz is President and COO at Guardant Health, Inc. and has ownership interest (including stock, patents, etc.) in the same. S.J. Hollingsworth has ownership interest in AstraZeneca stock. No potential conflicts of interest were disclosed by the other authors.

Authors’ Contributions
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): J. Lee, S.T. Kim, H. Lee, I. Kozarewa, J.I. Odegaard, J. Lee, S.T. Kim, S. Luke, Y.H. Lim, A.A. Talasaz, W.K. Kang
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