

**Clinical activity of transforming growth factor- β inhibitor vactosertib in combination
with imatinib in desmoid tumors: a multicenter phase Ib/II study**

Jin-Hee Ahn^{1†}, Jeeyun Lee^{2†}, Changhee Park^{3†}, Seung-Hoon Beom⁴, Seung Hyun Kim⁵,
Young Han Lee⁶, Kum-Hee Yun⁴, Jeung Eun Kim¹, Wooyeol Baek⁷, Yoon Dae Han⁸,
Sang Kyum Kim⁹, Hyang Joo Ryu⁹, Inkyung Jung¹⁰, JooHee Lee⁵, Hong In Yoon¹¹,
and Hyo Song Kim^{4*}

¹Department of Oncology, University of Ulsan College of Medicine, Asan Medical Center,
Seoul, Republic of Korea.

²Division of Hematology-Oncology, Department of Medicine, Samsung Medical Center,
Sungkyunkwan University School of Medicine, Seoul, Korea

³Department of Internal Medicine, Seoul National University Hospital, Seoul, Korea

⁴Division of Medical Oncology, Department of Internal Medicine, Yonsei Cancer Center,
Yonsei University College of Medicine, Seoul, Korea

⁵Department of Orthopedic Surgery, Yonsei University College of Medicine, Seoul, Korea

⁶Department of Radiology, Yonsei University College of Medicine, Seoul, Korea

⁷Department of Plastic Surgery, Yonsei University College of Medicine, Seoul, South Korea.

⁸Department of Surgery, Yonsei University College of Medicine, Seoul, Korea

⁹Department of Pathology, Severance Hospital, Yonsei University College of Medicine, Seoul,
Republic of Korea

¹⁰Division of Biostatistics, Department of Biomedical Systems Informatics, Yonsei University

College of Medicine, Seoul, Republic of Korea.

¹¹Department of Radiation Oncology, Yonsei Cancer Center, Yonsei University College of Medicine, Seoul

[†]Authors contributed equally

***Corresponding author:**

Hyo Song Kim, M.D., Ph.D.

Division of Medical Oncology, Department of Internal Medicine

Yonsei Cancer Center, Yonsei University College of Medicine

50 Yonsei-ro, Seodaemun-gu, Seoul 03722, Korea, 120-752

Tel: +82-2-2228-8124

E-mail: hyosong77@yuhs.ac

Running head: Vactosertib and imatinib combination for desmoid tumors

Abstract

BACKGROUND

To determine to the activity and safety of TGF- β inhibitor, vactosertib in combination with imatinib in patients with desmoid tumors.

PATIENTS AND METHODS

In this investigator-initiated, open-label, multicenter, phase Ib/II trial, patients with desmoid tumors not amendable to locoregional therapies (surgery and/or radiotherapy) or with disease progression following at least one treatment were enrolled. Participants were administered 400 mg imatinib daily in combination with vactosertib (5 days on and 2 days off, twice a day) every 28 days. In phase Ib, the vactosertib dose was set at 100 mg (level -1) and 200 mg (level 1) to determine the recommended phase II dose (RP2D). Phase II assessed the efficacy, with the primary endpoint being progression-free rate (PFR) at 16 weeks.

RESULTS

No dose-limiting toxicities were observed during the phase Ib, therefore RP2D was defined at doses of 400 mg imatinib daily in combination with 200 mg vactosertib. Of the 27 patients evaluated, 7 (25.9%) achieved a confirmed partial response and 19 (70.4%) were stable. The PFR at 16 weeks and 1 year were 96.3% and 81.0%, respectively. The most toxicities were mild to moderate myalgia (n=10, 37%), anemia (n=10, 37%), and nausea (n=9, 33.3%). Common grade 3-4 toxicities included neutropenia (n=6, 22.2%) and anemia (n=5, 18.5%).

CONCLUSIONS

Vactosertib and imatinib combination was well-tolerated, with promising clinical activity in patients with progressive, locally advanced desmoid tumors. This is the first study investigating a novel target agent, a TGF- β inhibitor, in this rare and difficult-to-treat desmoid tumor.

Translational Relevance

The transforming growth factor- β (TGF- β) response signature was known to be upregulated in desmoid tumors and combination of TGF- β inhibitor with imatinib showed synergistic antiproliferative activity. We conducted an open-label, phase Ib/II multicenter study to assess the activity and safety of TGF- β inhibitor vactosertib in combination with imatinib in patients with desmoid tumors. Treatment was well tolerable with 25.9% of objective response rate and 77.8% of progression-free after 2 years. In our exploratory analysis, canonical and non-canonical pathways related to TGF- β signaling were significantly enriched in responders whereas immune responses were enriched in non-responders. This study provided TGF- β inhibitor and imatinib showed promising efficacy with favorable safety warranting further investigation for desmoid tumor.

1 Introduction

2 Desmoid tumors and aggressive fibromatosis are fibroproliferative neoplasms that
3 originate from connective tissues (1, 2). These tumors are rare, with an incidence of 5-6 cases
4 per 1 million annually (2). Treatment selection depends on tumor aggressiveness and/or the
5 need for size reduction. Currently, active surveillance is the treatment of choice for
6 asymptomatic patients, and surgery is considered a treatment option for local control (3, 4).
7 With a local recurrence rate of approximately 30–70% (5, 6), a stepwise therapeutic approach
8 is recommended (7).

9 Various systemic therapeutic options, including tyrosine kinase inhibitors (TKIs) and
10 chemotherapeutic agents, have shown clinical benefits in patients with progressive or
11 symptomatic disease. Chemotherapeutic agents used in a low-dose regimen, such as
12 methotrexate and vinblastine, were also widely used in clinical practice, with relatively high
13 response rates (approximately 40%) (8, 9). Small studies utilizing cytotoxic chemotherapy
14 agents such as doxorubicin or pegylated liposomal doxorubicin have also shown clinical
15 benefits (10, 11). Although these therapies remain the standard, given their possible long-term
16 toxicity and the need for frequent visits for intravenous regimens, newer oral agents are being
17 actively investigated.

18 Recently, TKIs such as imatinib, sorafenib, and pazopanib have also been used
19 against desmoid tumors. Imatinib has been studied in several prospective trials and has
20 achieved partial response rates ranging from 6 to 16% (12-14). Sorafenib reported a
21 promising efficacy with 25% of response and 70% of symptom improvement (15). Additional
22 phase II trials have focused on pazopanib, which showed promising efficacy in contrast to
23 methotrexate plus vinblastine (16). Recently, nirogacestat, a γ -secretase inhibitor,
24 demonstrated promising activity in a phase III randomized trial (17). Despite the lack of
25 comparative studies and their lower toxicity, sorafenib and pazopanib have become the most

commonly used TKIs for the treatment of symptomatic and/or progressive desmoid tumors. Although sorafenib and pazopanib are effective, they commonly cause adverse effects and require dose modifications, even with low-dose administration. In addition, in patients who are non-responsive or progressive while receiving TKI therapy, further investigation of prospective oral agents is essential to prioritize other available options for desmoid tumors.

There is crosstalk between Wnt signaling and transforming growth factor- β (TGF- β) signaling during fibroblast activation, and TGF- β plays an important role in desmoid tumor cell growth and cell-cell interaction (18). Overexpression of TGF- β is commonly observed in desmoid tumors, indicating its crucial role in the development of fibromatosis (19). The TGF- β response signature, an indicator of specific TGF- β pathway activation in the tumor microenvironment, is upregulated in desmoid tumors, and a combination of a TGF- β inhibitor with imatinib showed synergistic antiproliferative activity in a patient-derived desmoid model (20). Vactosertib (TEW-7197, Medpacto, Seoul, South Korea) is a selective inhibitor of the serine/threonine TGF- β type I receptor kinase (ALK4/ALK5), and various clinical trials are ongoing for solid and hematologic malignancies (NCT03698825, NCT03732274, NCT03724851, NCT03143985, and NCT03666832). Based on the rationale for targeting TGF- β as a therapeutic strategies, we conducted an open-label, phase Ib/II multicenter study to assess the activity and safety of TGF- β inhibitor vactosertib in combination with imatinib in patients with desmoid tumors.

Methods

Patient Selection and Trial design

Patients enrolled in this study had histologically confirmed desmoid tumors as per the following inclusion criteria: (1) Patients ≥ 19 years, (2) not amenable for locoregional therapies (surgical resection or radiotherapy), or experienced disease progression following at least one standard therapy, (3) at least one measurable lesion according to Response Evaluation Criteria In Solid Tumours (RECIST) 1.1 (21), (4) Eastern Cooperative Oncology Group performance status of 0-1 and (5) adequate renal, hepatic, and cardiac function per protocol. Exclusion criteria were prior treatment with a TGF- β inhibitor and anticancer or radiation therapy < 2 weeks before enrollment.

The trial protocol was approved by the institutional review board of each center, and all patients provided written informed consent before enrollment, in accordance with the Declaration of Helsinki and the Guidelines for Good Clinical Practice (ClinicalTrials.gov identifier: NCT# NCT03802084). This is an investigator-initiated, open-label, multicenter, phase I/II trial (Supplementary Figure 1). Phase Ib assessed the safety and tolerability of imatinib (400 mg daily) in combination with vactosertib (5 days on/2 days off, twice a day) every 28 days. The vactosertib dose consisted of two levels: 100 mg (level -1) and 200 mg (level 1). Applying the classic 3+3 scheme, if none of the first three patients experienced dose-limiting toxicity (DLT) during level -1, three more patients were recruited at dose level 1. DLT was evaluated for 28 days and defined as grade ≥ 4 neutropenia or thrombocytopenia lasting 7 days, febrile neutropenia, or grade ≥ 3 non-hematological toxicity lasting > 4 days. The recommended phase II dose (RP2D) was defined as the dose level immediately below that at which $\leq 30\%$ of patients experienced a DLT. When no DLT was detected during the 1st cycle of level -1 patients, they were treated with a 200 mg dose of vactosertib from the 2nd

1 cycle. Treatment was continued until disease progression, unacceptable toxicities, or withdrawal of consent.

Study procedure; screening, evaluation and follow-up

Pretreatment evaluation consisted of taking a patient history, physical examination, blood chemistry, urinalysis, and a pregnancy test, if appropriate. Radiographic evaluation was performed by computed tomography or magnetic resonance imaging at baseline and every 8 weeks for 16 weeks and then every 16 weeks thereafter.

Peripheral blood samples were obtained for pharmacokinetic analyses as follows. During the vactosertib monotherapy period (day 1–5 of the 1st cycle, Supplementary Figure 1), blood was collected prior to administration on days 3, 4, and 5. In addition, on day 5, blood was collected at 0.5, 1.5, 3, 4.5, 8, and 12 h after administration. During the combination period, blood was collected prior to vactosertib administration on days 11 and 12. In addition, blood was collected 0.5, 1.5, 3, 4.5, 8, and 12 h after administration on day 12. Plasma vactosertib concentration was measured using a validated LC-MS/MS method. Pharmacokinetic data were analyzed by non-compartmental analysis using WinNonlin software version 8.3.4 (Pharsight Corporation, Sunnyvale, CA, USA) and parameters were calculated as described previously (22). Primary parameters were log-transformed and analyzed by ANOVA with a mixed-effects model. In order to compare these parameters, point estimates and 90% CIs for the geometric mean ratios (combination therapy/monotherapy) of the log-transformed $C_{\max,ss}$ and $AUC_{\tau,ss}$ were also presented

End points and assessment

The primary endpoint of the trial was the proportion of patients who had not progressed at 16 weeks (PFR at 16 weeks), defined as the percentage of patients remaining alive and progression-free at 16 weeks as per RECIST 1.1. The secondary endpoints included the objective response rate (ORR), disease control rate (DCR), progression-free survival (PFS), overall survival (OS), safety profile using the NCI CTCAE v4.03, and exploratory biomarker analysis. PFS was defined as the time from the start of treatment to the date of disease progression or death, and OS was measured from the start of treatment to the date of death from any cause. The ORR was calculated as the percentage of patients experiencing a confirmed complete response (CR) or partial response (PR), and DCR was calculated as response rate + stable disease, according to the RECIST 1.1 guidelines. Statistical analyses were performed using SPSS version 26 (IBM Corp., Armonk, NY, USA) and GraphPad Prism version 6 (GraphPad Software, La Jolla, CA, USA) software.

Sample size and statistical analysis

The phase Ib stage was designed to complete up to two dose cohorts of vactosertib in three patients to establish the RP2D. Applying Simon's minimax 2-stage design, the null hypothesis ($P_0 \leq 60\%$) would not warrant further investigation and the alternative hypothesis ($P_1 \geq 80\%$) would warrant further investigation after treatment completion. With $\alpha=0.1$ (to account for type I error) and $\beta=0.2$ (to account for type II error), we assumed that further testing would be warranted if ≥ 7 cases were progression-free at 16 weeks out of the initial 11 patients who participated in the first stage (including RP2D in phase I). If at the end of recruitment ≥ 18 of 24 evaluable patients were progression-free at 16 weeks of treatment, an additional investigation would be considered. Allowing for a follow-up loss rate of 10 %, the total sample size is expected to be 27 patients in the phase I/II stages. The assessable population included all patients who met the eligibility criteria and had received at least one

cycle of the RP2D of vactosertib.

Correlative science

Tumor tissues were obtained from 11 patients prior to treatment, 10 of whom had successful RNA-sequencing analyses. Briefly, total RNA was extracted and purified from tumor samples using the ReliaPrep FFPE Total RNA Miniprep System (Promega, Madison, WI, USA) and assessed using a BioAnalyzer2100 instrument (Agilent Technologies, Santa Clara, CA, USA). RNA libraries were constructed using the SMARTer Stranded Total RNA-Seq Kit v2 (Pico Input Mammalian kit, Illumina, San Diego, CA, USA) and high-quality libraries were pooled and sequenced using the Illumina NovaSeq6000 platform (Illumina, San Diego, CA, USA), with 150 bp paired-end reads according to the manufacturer's protocols.

RNA-sequencing data were normalized using the trimmed mean of M values algorithm (23), and the single sample gene set enrichment analysis was performed to estimate the enrichment score in hallmark pathways and TGF- β response signatures (24-26). We also performed gene set enrichment analysis (GSEA) to investigate the potentially enriched pathways in responders and non-responders using RECIST 1.1. In the GSEA, a nominal p value < 0.2 was considered as a potentially enriched pathway. Then, we selected the genes that were significantly highly expressed in either responders or non-responders with fold change cutoffs > 1.25 or < 0.8 , and p value by t-test < 0.05 , and performed gene ontology analysis (27). Finally, we extracted the genes that were associated with significantly enriched gene ontology and defined the response signature score as the sum of the expressions of genes enriched in responders subtracted by the sum of the expressions of genes enriched in non-responders. The Wilcoxon rank-sum test was used to compare differences between signature scores. Part of RNA-sequencing data was previously published in our preclinical

1 study (20).

2

Data availability

All data generated in this study are available within the article and supplementary data; Raw sequences were deposited in the European Nucleotide Archive (ENA) at the European Molecular Biology Laboratory- European Bioinformatics Institute (EMBL-EBI) data portal under bioproject accession number PRJEB71995. Additional individual patient data and Sequencing data not publicly available due to patient privacy requirements but are available upon reasonable request from the corresponding author (Hyo Song Kim, hyosong77@yuhs.ac) without any patient-identifying information and the data should be used for research purpose only.

Results

Demographic and Clinical Characteristics

Between March 2019 and August 2021, 29 participants were recruited, and 28 received the trial treatment (CONSORT, Supplementary Figure 2). Of these, 27 underwent radiographic imaging and were evaluable for per-protocol analysis. The baseline characteristics of the patients are shown in Table 1. Median primary tumor size was 10.0 cm (range of 2.8–22.6 cm) and half of cases were larger than 10 cm (48.1%). The most frequent primary site was the abdomen (n=12, 44.4%), which consisted of the abdominal wall (n=5) and mesentery (n=7). The majority (85.2%) of patients had previously undergone treatment (surgery [n=16, 59.3%], radiotherapy [n=5, 18.5%], and/or systemic therapy [n=19, 70.4%]).

Treatment Efficacy

As of the data collection cutoff date (October 1, 2022), 14 patients remained in

1 treatment, with a median follow-up of 20.7 months. Of the 27 patients evaluated, 7 (25.9%)
2 achieved a confirmed partial response (cPR) and 19 (70.4%) achieved stable disease,
3 resulting in a DCR of 96.3% (Figure 1A). The median time to cPR was 7.5 months (range of
4 3–13.3 months), and the median duration of response was 14.9 months (range of 11.2–33.8
5 months; Figure 1B). Representative examples of cPR include (i) a 33-year-old woman who
6 had experienced disease progression following previous treatment with imatinib and
7 sorafenib monotherapies demonstrated cPR with the combination of vactosertib and imatinib
8 (Figure 1C) and (ii) a 59-year-old man had a recurrent tumor extending into the 2–4th
9 intercostal space after surgical resection. After 6 months of treatment, the patient experienced
10 cPR (Figure 1D).

11 The median PFS was not reached, and the PFR at 16 weeks and 1 year were 96.3%
12 and 81.0%, respectively (Figure 2A). Twenty-one patients (77.8%) remained progression-free
13 after 2 years of treatment. We analyzed outcomes based on previous treatment with surgery,
14 radiotherapy, and chemotherapy. Among these, only previous radiotherapy was significantly
15 associated with PFS ($p=0.029$, Figure 2B), whereas systemic treatment and surgery were not
16 (Supplementary Figure 3). Finally, treatment-naïve patients had longer PFS than those with 1,
17 2 or 3 prior treatments (1 year PFS 100% vs 90% vs 25%, $p=0.017$, Figure 2C).

18 **Safety**

19 In general, vactosertib and imatinib combination therapy was well-tolerated (Table 2).
20 No DLTs were observed during the phase Ib, therefore RP2D was defined at doses of 400 mg
21 imatinib daily in combination with 200 mg vactosertib. Regarding vactosertib, one dose
22 reduction (100 mg BID) was performed in eight (29.3%) patients, and two (7.4%) patients
23 stopped vactosertib. For imatinib, eight (29.3%) patients had dose reduction by one level (300
24 mg/day), one (3.7%) patient has dose reduction by two levels (200 mg/d), and one (3.7%)
25

patient stopped imatinib (Figure 2D). The most common toxicities were myalgia (n=10, 37%), anemia (n=10, 37%), and nausea (n=9, 33.3%). Grade 3/4 events occurring at frequencies of >5% included neutropenia (n=6, 22.2%) and anemia (n=5, 18.5%). The toxic effects leading to premature dropout from treatment were as follows (n=3): grade 3 macular-popular rash, recurrent grade 3 anemia, and grade 3 perineal infection. The mean (\pm SD) dose intensity was 78.7% (SD \pm 18.8, 95% CI 71.3-86.1) for vactosertib and 84.5% (SD \pm 13.8, 95% CI 79.1-89.9) for imatinib.

Pharmacokinetics

Vactosertib was rapidly absorbed at 100 mg and 200 mg BID, with maximum plasma concentrations occurring at a median T_{max} of 0.5–3.0 hours. After reaching C_{max} , the plasma concentrations of vactosertib appeared to decline in a generally multiphasic manner with a similar terminal elimination half-life ($t_{1/2}$). The mean $t_{1/2}$ of vactosertib was between 2.2–2.8 hours with or without imatinib and median t_{max} (1.5 h) was consistent across both cohort of 100 and 200 mg (Supplementary Table 1). Compared to vactosertib monotherapy (day 5), the C_{max} and AUC of vactosertib at 100 mg and 200 mg BID doses (day 12) were increased in the presence of imatinib (Table 3, Figure 3A and B). The estimates of the geometric mean ratios of vactosertib $AUC_{\tau,SS}$ and $C_{max,SS}$ were 1.6845 (90% CIs, 1.3199-2.1499) and 1.4451 (90% CIs, 1.0005-2.0872), respectively. $AUC_{\tau,SS}$ of vactosertib was statistically different between the vactosertib monotherapy, and its combination therapy with imatinib ($P=0.0060$, Supplementary Table 2).

Correlative Analysis

We estimated the normalized enrichment score to identify pathways potentially associated with treatment response. In GSEA, pathways related to DNA repair, oxidative phosphorylation, MYC targets, glycolysis, unfolded protein response, peroxisome, and epithelial mesenchymal transition were enriched in responders, whereas immune responses such as TNF- α signaling, IL6-JAK-STAT3 signaling, allograft rejection, and inflammatory response were enriched in non-responders (Figure 4A, Supplementary Figure 4A and 4B). Among these pathways, Gene Ontology analysis showed that mitochondrial electron transport related to oxidative phosphorylation had a significantly enriched gene ontology term responders (related genes: *NDUFB6*, *NDUFA8*, *NDUFC2-KCTD14*, and *NDUFB3*) and inflammatory response (related genes: *MMP25*, *AGER*, *NFKBID*, *TNF*, *HPSE*, and *TLR9*) had a significantly enriched gene ontology relative to non-responders (Figure 4B). With these 10 genes, responders had significantly higher response signature scores than the non-responders ($p=0.010$, Figure 4C). TGF- β response signatures (fibroblasts, T-cells, macrophages, and endothelial cells) (23) were not significantly different between responders and non-responders (Figure 4D).

Discussion

This prospective clinical trial provides meaningful insight into the activity of vactosertib and imatinib combination therapy in patients with desmoid tumors. To the best of our knowledge, this is the first study to report a novel targeting agent, a TGF- β inhibitor, in desmoid tumors. Further investigation is needed to confirm the clinical benefits of incorporating this therapy into standard treatment regimens for this rare tumor.

A wide spectrum of treatments are available for desmoid tumors. However, due to the rarity of desmoid tumors, a few prospective trials have addressed the chemotherapeutic options. Because of the toxic side effects of cytotoxic drugs, such as liposomal doxorubicin, vinblastine, and methotrexate (9, 10, 28), potential alternative non-cytotoxic agents are highly desired. TKIs, such as imatinib, sunitinib, and sorafenib, have achieved partial success. As a first-generation TKI, imatinib has modest efficacy (approximately 10% ORR), with favorable toxicity profiles (12, 14, 29). In the DeFi trial, a γ -secretase inhibitor showed significant efficacy, resulting in a 71% reduction in the risk of disease progression or death compared with placebo (17). Multitargeted TKIs, such as sorafenib (ALLIANCE A091105 trial) and pazopanib (DESMOPAZ trial), have significant activity (33% ORR to sorafenib, 27% ORR to pazopanib) and over 80% progression-free rates after one year. In our study, the notable efficacy (25.6% ORR and 81% progression-free rate after one year) of vactosertib and imatinib was consistent with that of previously studied multitargeted TKIs. In addition, it is noteworthy that the radiological response continuously improved, with a median of seven and a half months to the best response in our study. Compared to the ALLIANCE A091105 (36%) and DeFi (61%) trials (15, 17), over 70% of the participants in our study had already been treated with systemic therapy, with 40% being heavily treated patients (≥ 2 prior treatments). Furthermore, patients with large tumors were enrolled in our study, with a 10 cm median total

size of target lesions, compared to 8.4 cm in the previous sorafenib study. In addition, synergistic efficacy was also seen in imatinib-refractory patients (n=4), with one cPR and two patients achieving long-term SD.

In this trial, the TGF- β inhibitor vactosertib, in combination with imatinib, was generally well tolerated. Mild constitutional symptoms, including myalgia and fatigue, were the major adverse events. Grade 3–4 neutropenia or anemia occurred in ~20% of patients but were manageable without clinically significant complications. In the ALLIANCE A091105 trial, lower doses of sorafenib were administered at 400 mg daily, which is 50% of the licensed dose, while the DESMOPAS trial used the standard dose of pazopanib (800 mg daily). Dose modification and discontinuation due to toxicities (29.5% and 7.4% respectively for vactosertib, 25.9% and 3.7% respectively for imatinib) were much lower for our study treatment than the ALLIANCE A091105 (31% dose modification and 20% discontinuation) and DESMOPAS trials (73% dose reduction and 8% discontinuation). Compared to pazopanib and sorafenib, less toxicities may enable to achieve long term stabilization via less frequent dose reduction and/or withdrawal. Additionally, the PFS of treatment-naïve patients in our study is very promising, not only as a salvage therapy, but also as a front-line therapy alternative. Furthermore, although randomized trials for rare diseases are generally difficult, we were able to rapidly compile the necessary clinical data based on a multicenter study. This was made possible by the high unmet need for novel therapeutic options for desmoid tumors. In our study, the majority of patients had been treated for more than 1 year, but the appropriate duration of combination treatment remains to be determined. Four patients categorized as long-term responders are in the resting period, and long-term follow-up results for those may elucidate more information on appropriate treatment durations. However, our trial is still a phase Ib/II trial with preliminary data. Therefore, our clinical findings should be

cautiously interpreted and validated in future randomized trials that compare our combination therapy with other agents in a front-line setting.

Furthermore, the possibility of an interaction between vactosertib and imatinib was evaluated in our pharmacokinetic study. In the phase I study of vactosertib (NCT02160106), 29 patients were treated with vactosertib once daily at a dose range of 30–340 mg, and six patients were treated with 200 mg twice daily (5 days with 2 days off). Based on this study, safety and efficacy evaluation of vactosertib 200 mg twice daily was incorporated in various tumors, including urothelial carcinoma (NCT04064190) and multiple myeloma (NCT03143985). In our study, although our concerns for the drug-drug interaction that may increase vactosertib concentration, the pharmacokinetic properties observed here were comparable to the value in other trials (30), and clinical significance may be limited considering the favorable toxicity profiles in the present study.

Despite being the recommended standard treatment for many cancers, the underlying mechanisms of multi-tyrosine kinase inhibitors remain unknown. In the DESMOPAZ study, PDGF receptor-like protein and thrombospondin-4 were highly expressed. Thrombospondin-4, a proangiogenic factor, stimulates tumor growth via TGF- β signaling (31). In addition, TGF- β and connective tissue growth factor, downstream effectors of Wnt/ β -catenin signaling, are highly expressed in desmoid tumors (18, 32). Serine protease inhibitors, including plasminogen activator inhibitor-1, were upregulated in desmoid tumors (33) and increased tumor growth and invasion in a TGF- β -dependent manner (33). Therefore, TGF- β may be a potential therapeutic target for desmoid tumors, and combined treatment with TGF- β and imatinib shows efficacy and synergy compared to monotherapy in preclinical models^{19, (34)}. Indeed, our clinical study validated that combination treatment was effective and well-tolerated for desmoid tumors.

1 In our exploratory analysis, the TGF- β signaling enrichment score was not
2 statistically significant. However, various canonical and non-canonical pathways related to
3 TGF- β signaling were significantly enriched in responders. Upon DNA damage, ATM
4 activates TGF- β signaling, which subsequently maintains genomic stability through DNA
5 repair pathways (35, 36). TGF- β and unfolded protein response pathways are associated with
6 the secretion of extracellular matrix proteins and epithelial-mesenchymal transition (37, 38).
7 Meanwhile, considering the immunosuppressive role of TGF- β signaling within the tumor
8 microenvironment (39), tumors enriched with immune responses may not depend on TGF- β
9 signaling and are not responsive to TGF- β inhibitors, as shown in our study. Therefore,
10 further investigation of TGF- β inhibitors with correlative markers in large cohorts might help
11 elucidate the efficacy and mechanisms underlying clinical responses.

12 This study had several limitations. The study design did not include a comparison of
13 the direct effects of the combined regimen with those of other agents. Considering the high
14 spontaneous disease regression (28%) and ORR in the placebo group (20%) (15, 40), the lack
15 of randomization in our study made it difficult to determine definitive efficacy. Additionally,
16 progression according to RECIST was not mandatory for inclusion in our study. However,
17 85.2% of the participants in our study had previously been treated and enrolled after disease
18 progression, according to RECIST criteria. All treatment-naïve patients were enrolled in this
19 trial after disease progression during active surveillance. Based on recent trials (15, 17),
20 further placebo-controlled randomized trials should be considered for primary efficacy.
21 Considering the main challenge of randomized trials in rare cancers, crossover to imatinib
22 monotherapy in the placebo group may provide other points as secondary outcomes.

23 In conclusion, the combination of TGF- β inhibitor and imatinib showed promising
24 efficacy with favorable safety profile in the desmoid tumors. Based on the promising

1 response rates, further investigation with a randomized trial is needed to confirm these
2 findings. This novel therapeutic approach may provide a silver lining for this rare and
3 disabling disease.

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H.S.K. contributed to the conception and design of the study and obtained research funding. H.S.K., J.L, Y.H.L., S.H.K., W.B., Y.D.H., J.E.K. H.I.Y, and J.H.A. treated study patients. S. K.K. and H.J.R. conducted all the pathological review and analyses. C.P and I.J. conducted genomic and transcriptomic data analysis and statistical tests. H.S.K. wrote the manuscript. All authors discussed and approved the final manuscript.

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Figure legends

Figure 1. Response rate to vactosertib and imatinib combination therapy

(A) Waterfall plot representing reduction of tumor size as a percentage of maximum tumor size after treatment, as assessed according to RECIST 1.1 criteria

(B) Spider plots of the per-subject percentage change in the sum of target lesions.

(C and D) Representative pre- and post-treatment scans of patients #Y06 and #Y01.

Figure 2. Progression free survival and time on treatment after combination therapy

Kaplan–Meier analysis of progression-free survival for all patients (A) and according to previous radiotherapy (B) and number of prior modalities (C). Dotted lines represent 95% confidence intervals.

(D) Swimmer plots for the time of therapy and duration of the response. Dose modification of vactosertib and imatinib are also indicated. The x-axis represents the duration of treatment for each patient.

Figure 3. Pharmacokinetic model of vactosertib with or without imatinib.

Mean (\pm standard deviation) plasma concentration-time profile of vactosertib with or without imatinib at steady state after dose adjustment of vactosertib 100 mg BID (A) and 200 mg BID (B)

Figure 4. Transcriptomic correlates of the clinical response to vactosertib combined with imatinib.

(A) Normalized enrichment score of pathways by gene set enrichment analysis. Positive values (red bars) indicate pathways enriched in responders and negative values (blue bars) indicate pathways enriched in non-responders.

(B) Heatmap of the expression of significantly differentially expressed genes among the significant pathways between responders and non-responders.

(C) Boxplot showing response signature scores according to response group.

(D) TGF- β response signature (TBRS) differences between responders and non-responders. *p* values by the Mann-Whitney test are shown. F-, fibroblast; T-, T cell; Ma-, macrophage; End-, endothelial cell.

Table 1. Baseline characteristics

Characteristics	Total (n, %)
Age (Median, range)	33 (21-60)
Gender	
Male	9 (33.3%)
Female	18 (66.7%)
ECOG PS	
0	18 (66.7%)
1	9 (33.3%)
Primary tumor site	
Head and neck	1 (3.7%)
Thorax	1 (3.7%)
Abdomen	12 (44.4%)
Extremity	8 (29.6%)
Spine and pelvic girdles	3 (11.1%)
Shoulder	2 (7.4%)
Size (Median, range)	10 (2.8-22.6)
≤ 5 cm (T1)	7 (25.9%)
5-10 cm (T2)	7 (25.9%)
>10 cm (T3)	13 (48.1%)

Presence of risk factors	
FAP	5 (18.5%)
Prior surgery	1 (3.7%)
Prior surgery	
No	11 (40.7%)
Yes	16 (59.3%)
Prior radiotherapy	18 (38.3%)
No	22 (81.5%)
Yes	5 (18.5%)
Mutation type (n=13)	
<i>CTNNB1</i> T41A somatic	5 (38.5%)
<i>CTNNB1</i> S45F somatic	4 (30.8%)
<i>APC</i> somatic	2 (15.4%)
<i>APC</i> germline	1 (7.7%)
No mutation	1 (7.7%)
Systemic therapy (n=19)	
NSAIDS	11
Hormone	6
Imatinib	4
Methotrexate and vinblastine	2

Sunitinib	2
Dacarbazine	1
Methotrexate	1

Table 2. Common Adverse Events

Event	Grade (n, %)				All	(%)
	1	2	3	4		
General						
Myalgia	8 (29.6)	2 (7.4)	10	37.0
Fatigue	4 (14.8)	2 (7.4)	6	22.2
Edema	7 (25.9)	7	25.9
Headache	4 (14.8)	2 (7.4)	1 (3.7)	..	7	25.9
Fever	4 (14.8)	4	14.8
Insomnia	2 (7.4)	2	7.4
Arthralgia	2 (7.4)	2	7.4
Urticaria		1 (3.7)			1	3.7
Gastrointestinal and genitourinary						
Diarrhea	6 (22.2)	6	22.2
Oral mucositis	3 (11.1)	1 (3.7)	.	.	4	14.8
Nausea	9 (33.3)	9	33.3
Vomiting	2 (7.4)	1 (3.7)			3	11.1
Hematuria	4 (14.8)	4	14.8
Dyspepsia	5 (18.5)	1 (3.7)	.	.	6	22.2

Abdominal pain	2 (7.4)	2 (7.4)	1 (3.7)	.	5	18.5
Skin and subcutaneous						
Rash	4 (14.8)	3 (11.1)	1 (3.7)	.	8	29.6
Paronychia	.	1 (3.7)	.	.	1	3.7
Perineal infection	2 (7.4)	2 (7.4)	1 (3.7)	.	5	18.5
Investigations						
Neutropenia	.	2 (7.4)	5 (18.5)	1 (3.7)	8	29.6
Anemia	.	5 (18.5)	5 (18.5)		10	37.0
Creatinine elevation	3 (11.1)	3	11.1
ALT increased	1 (3.7)	1	3.7
Proteinuria	.	.	1 (3.7)	.	1	3.7
Reproductive system						
Menorrhagia	4 (14.8)	.		.	4	14.8

Table 3. Comparison of pharmacokinetic parameters of vactosertib monotherapy and combination therapy

		AUC_{inf} (h*ng/mL)	C_{max} (ng/mL)	T_{max} (h)	T_{1/2} (h)
100 mg BID	Monotherapy (day 5)	2905 ± 1313	672 ± 181	1.5 [1.5-1.5]	2.5 ± 1.1
	Combination (day 12)	4888 ± 1946	1129 ± 618	1.5 [0.5-3.0]	2.8 ± 0.6
200 mg BID	Monotherapy (day 5)	6033 ± 3882	1973 ± 1011	1.5 [1.5-3.0]	2.2 ± 0.2
	Combination (day 12)	8857 ± 2625	2397 ± 571	1.5 [0.5-1.5]	2.5 ± 0.8

Figure 1

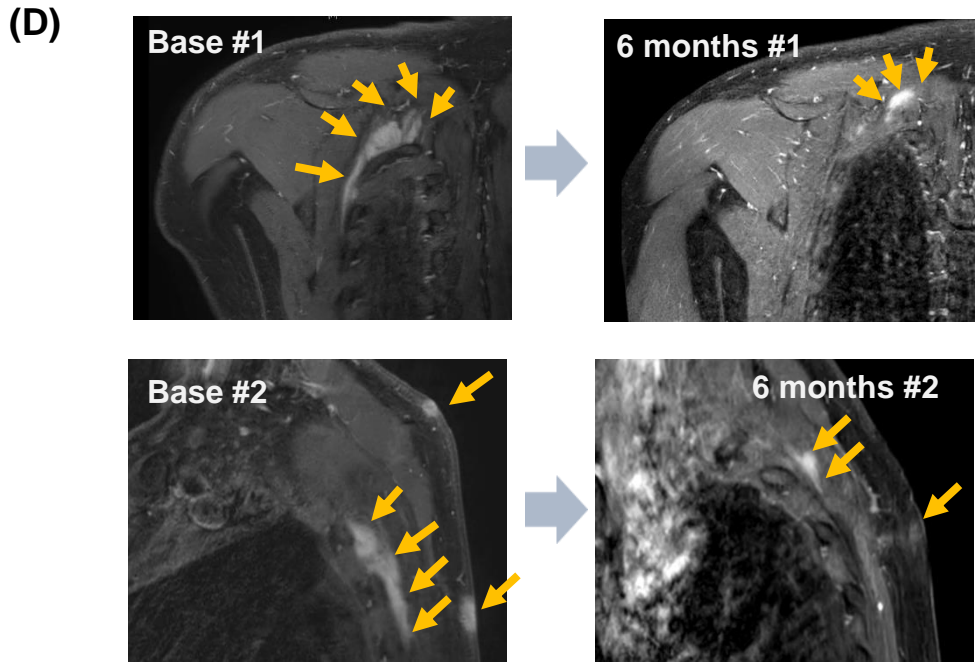
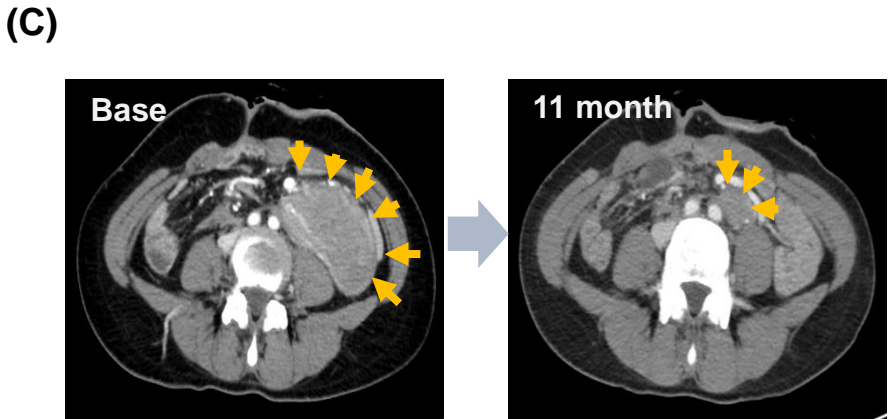
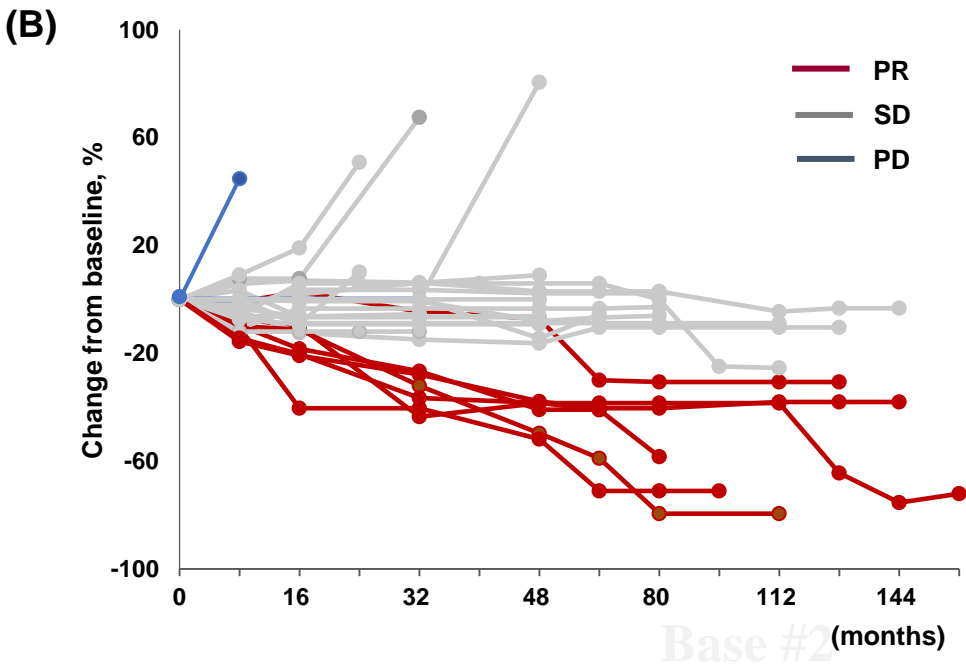
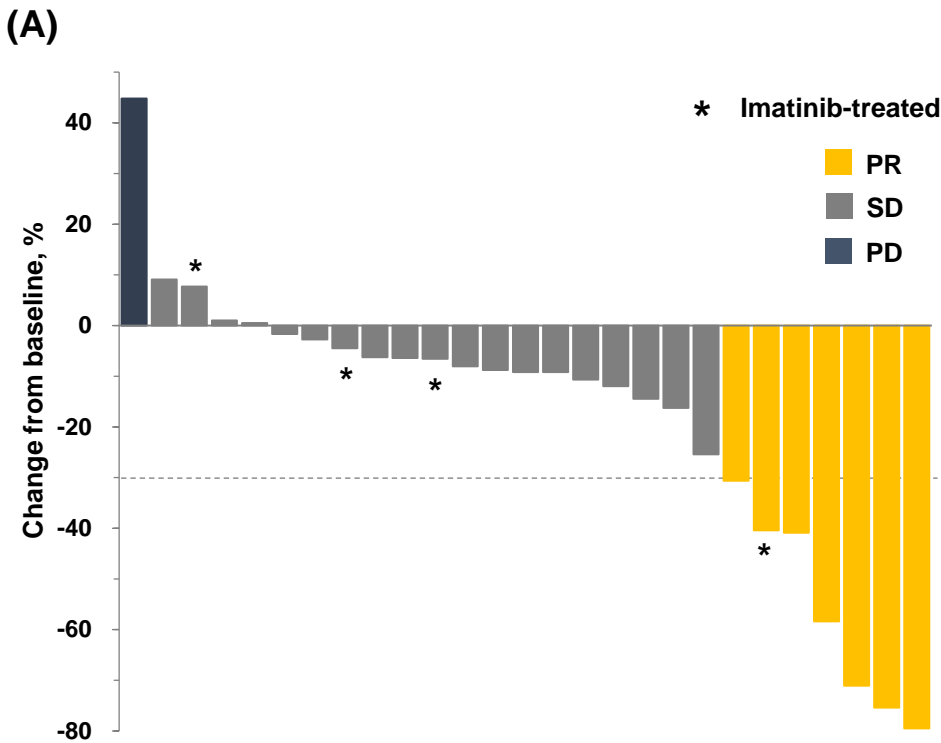


Figure 2

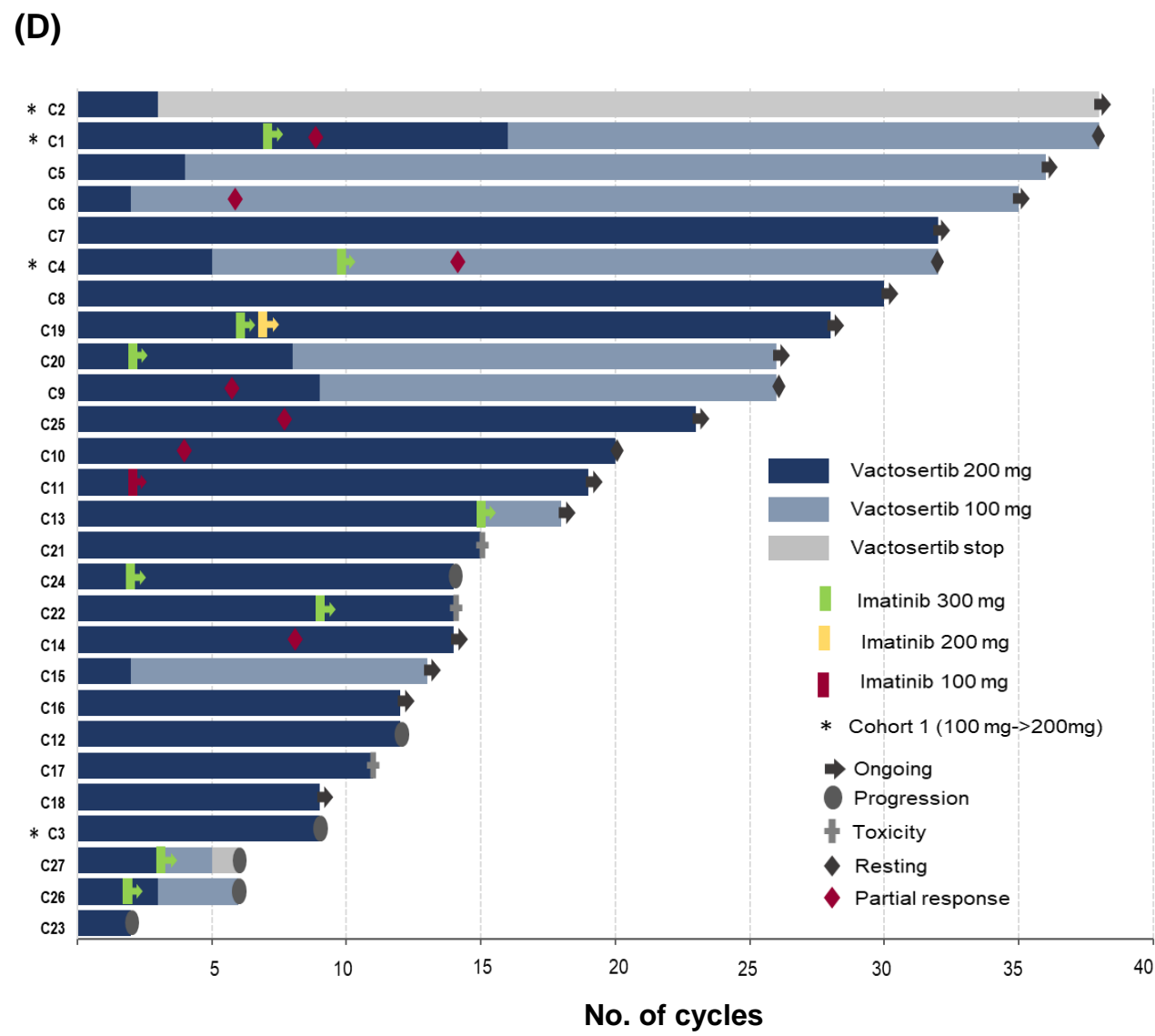
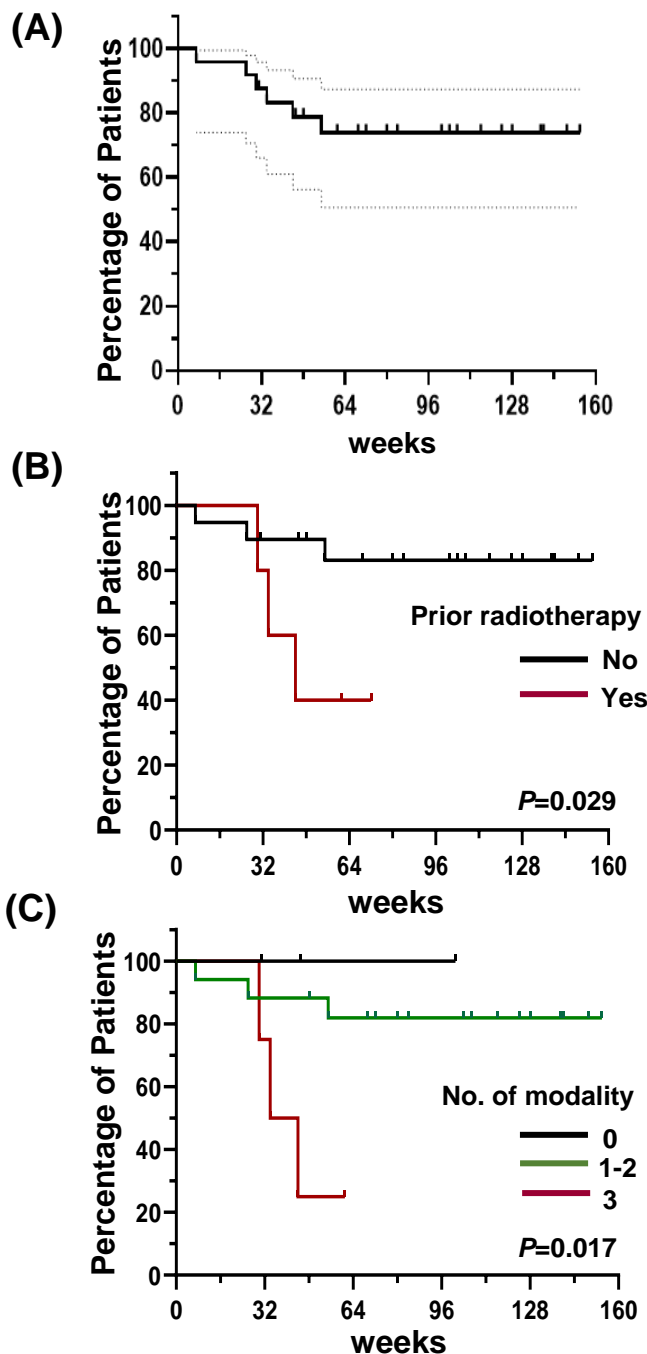


Figure 3

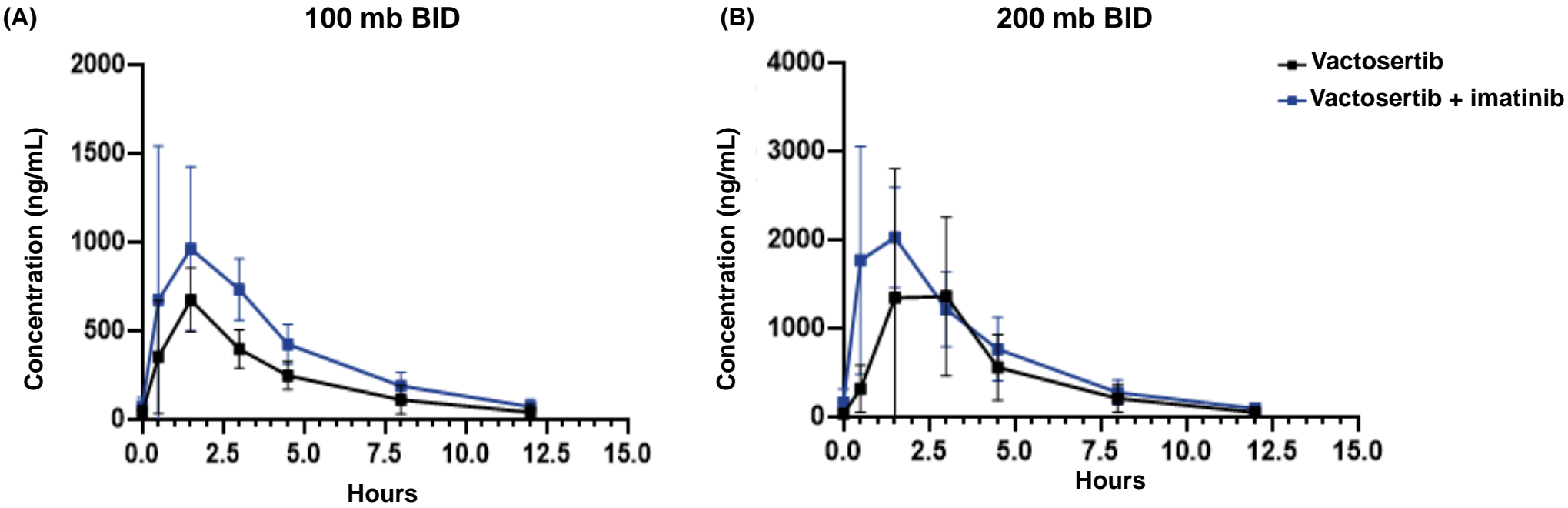


Figure 4

