A Phase 1b/2 Clinical Study of Onvansertib in Combination with FOLFIRI/Bevacizumab Revealed a New Role of PLK1 in regulating the Hypoxia Pathway in *KRAS*-mutant Colorectal Cancer

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Background

***KRAS*-mutant metastatic colorectal cancer (mCRC):**

- Represents ~50% of mCRC patients and have poorer prognosis than *RAS* wild-type patients.
- First- and second-line treatments are chemotherapy (FOLFIRI/FOLFOX) ± bevacizumab (Bev).
- Second-line regimens have limited efficacy:
  - ORR: 5%-10%, median PFS: ~6 months, median OS: ~12 months.[1-2]

Onvansertib: a promising therapeutic option for *KRAS*-mutant mCRC:

- Oral and highly selective PLK1 inhibitor.
- Demonstrated potent activity in CRC preclinical models as single agent and in combination with irinotecan.[3-5]

Phase 1b/2 study of onvansertib + FOLFIRI/Bev (NCT03829410):

- Patients: mCRC with *KRAS* mutation who failed or were intolerant to first-line treatment of fluoropyrimidine and oxaliplatin with or without Bev.
- Treatment (28-day cycle): onvansertib (Days 1-5 and 15-19) in combination with FOLFIRI/Bev (Days 1 and 15).
- Phase 1b demonstrated safety and promising efficacy[4]:
  - Onvansertib RP2D was established at 15 mg/m^2.
  - ORR was 44%, median PFS 12.6 months and median duration of response 9.5 months.

Here we explored response biomarkers to onvansertib + FOLFIRI/Bev therapy in the Phase 1b/2 study and their associated biology.

References: 1. Giessen et al., Acta Oncologica 2015, 54: 187-193; 2. Bennouna et al., Lancet Oncol. 2013; 14: 29–37; 3. Valsasina et al., Mol. Cancer Ther. 2012, 11:1006-16; 4. Ahn et al., Clin. Cancer Res. 2024; 5. Kopetz et al., Ann Oncol. 2022, 33(7):S704.

Results

1. Patient Treatment and Disposition

- Between JUL-2019 and OCT-2022, 68 patients were enrolled in 7 sites in the U.S., including 53 patients treated at the RP2D.
- As of 29-JAN-2024 (cut-off date), all patients have completed treatment and follow-up. Median follow-up was 7.1 months (range, 0.4-30.3).
- Reasons for discontinuation were progressive disease (n=40, 59%), pursue curative surgery (n=13, 19%), patient's choice (n=8, 12%), adverse event (n=6, 9%), transition to extended access program (n=1, 1%).

2. Clinical Activity

Table 1. Efficacy of onvansertib + FOLFIRI/bevacizumab.

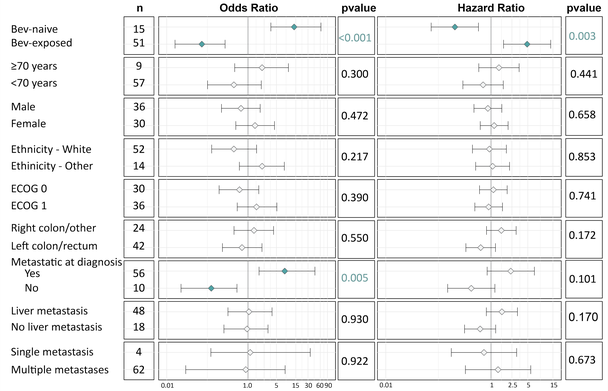
Patients (n)	ORR (%)	DCR (%)	mPFS [CI] (months)	mDOR [CI] (months)
66[a]	28.8	90.9	9.8 [7.5, 12.6]	11.7 [9.0, NR]
53[b]	26.4[c]	92.5	8.4 [5.8, 12.6]	11.7 [9.0, NR]

a. all onvansertib doses, b. onvansertib RP2D, c. all patients had confirmed responses. Patients who received at least 1 cycle of treatment were included in the analysis. Radiographic response determined per RECIST v1.1. **ORR:** overall response rate, include unconfirmed responses. **DCR:** disease control rate, include complete response, partial response and stable disease. **mPFS:** median progression-free survival. **mDOR:** median duration of response, defined as time between first response and progression. **NR:** not reached. **CI:** 95% confidence intervals.

3. Bev-naïve Patients Exhibit Superior Clinical Benefit to Onvansertib + FOLFIRI/Bevacizumab

- A subgroup analysis of baseline characteristics identified superior clinical benefit to onvansertib + FOLFIRI/bevacizumab in patients who did not receive bevacizumab in the first-line setting (Bev-naïve) compared to patients who received bevacizumab in first-line treatment (Bev-exposed).
- Bev-naïve patients had significantly greater ORR (OR=13.64, p<0.001) and longer PFS (HR=0.21, p=0.003) than Bev-exposed patients.
- There was no evidence of differences in treatment benefit (ORR and PFS) for the other subgroups.

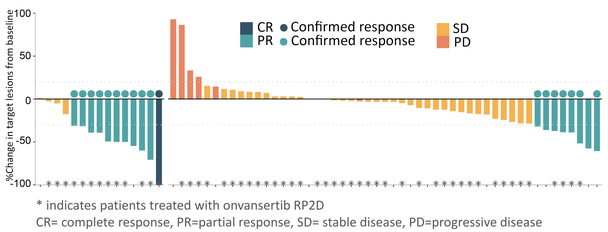
Figure 1. Subgroup analysis of baseline characteristics (n=66).



Odds ratios (OR) are based on the comparison of ORR; OR>1 indicates higher likelihood of response.
Hazard ratios (HR) are based on the comparison of PFS; HR<1 indicates lower likelihood of progression.

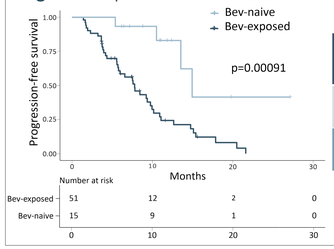
Figure 2. Waterfall plot of best radiographic response (n=66).

Bev-naïve	Bev-exposed
ORR 73.3% (95%CI 44.9-92.2)	**ORR 15.7%** (95%CI 7.0-28.6)



* indicates patients treated with onvansertib RP2D
CR= complete response, PR=partial response, SD= stable disease, PD=progressive disease

Figure 3. Kaplan-Meier curves of PFS.



	Patients (n)	mPFS [CI] (months)
Bev-naïve	15	14.9 [13.5-NR]
Bev-exposed	51	7.8 [5.7-9.8]

NR: not reached.
CI: 95% confidence intervals.

4. Onvansertib + Bevacizumab Inhibit Tumor Growth and Angiogenesis in *KRAS*-mutant Colorectal Cancer Xenograft Models

- Onvansertib + bevacizumab effectively inhibited tumor growth in 3 *KRAS*-mutant CRC xenograft models, resulting in tumor regression or stasis. Combination treatment had significantly superior antitumor activity compared to the monotherapies.
- Onvansertib reduced tumor vascularization, and onvansertib + bevacizumab treatment resulted in greater decrease in tumor vascularization.

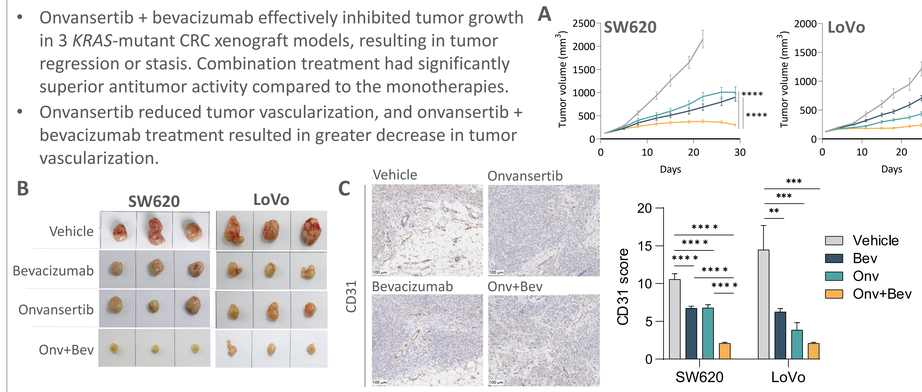
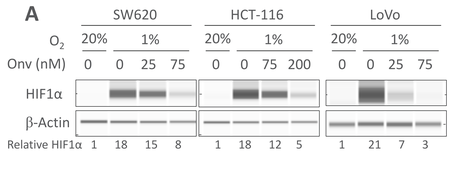


Figure 4. Xenografts were treated with onvansertib (Onv, oral, QD, 45 mg/kg), bevacizumab (Bev, IP, BIW, 1mg/kg for LoVo, 5 mg/kg for SW620 and HCT116) for 30-33days. **A.** Mean tumor volumes ± SEM (n=8-9/group). Tumor volumes were compared at end-of-study (EOS) by One-way ANOVA. **B.** Tumors were photographed at EOS. **C.** Tumors were stained with anti-CD31 antibody, representative images of the SW620 tumors are shown. CD31+ vessels were counted manually for each tumor (5 fields/tumor). Means ± SEM are shown and were compared by One-way ANOVA. **p<0.01,***p<0.001, ****p<0.0001.

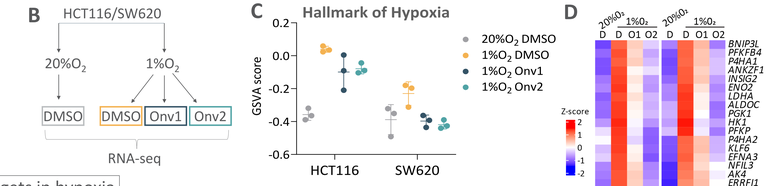
5. Onvansertib Inhibits the Activation of the Hypoxia Pathway via the Regulation of HIF1α

The hypoxia-inducible factor 1α, HIF1α, is stabilized under low level of oxygen and promotes the gene expression of downstream targets resulting in angiogenesis, metabolic changes and survival/proliferation of tumor cells.

Onvansertib inhibits HIF1α protein expression in hypoxia



Onvansertib inhibits the activation of the hypoxia pathway at the transcriptional level



D=DMSO
O1/Onv1=Onvansertib dose 1
O2/Onv2=Onvansertib dose 2

siPLK1 inhibits the expression of HIF1α and its downstream targets in hypoxia

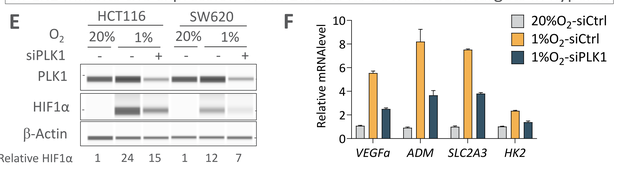
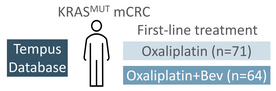


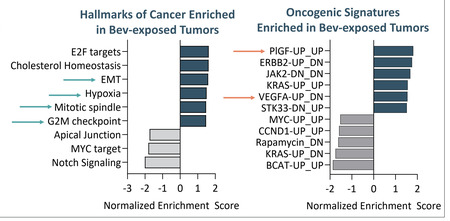
Figure 5. PLK1 was inhibited by onvansertib (A-D) or siPLK1 (E-F) for 20h and then cells were exposed to hypoxia for 4h. **A,E**: Immunoblot, HIF1α protein levels were normalized to β-actin and 20%O_2 control sample. **B**. Study design of the RNA-seq experiment. **C**. Expression data were transformed into gene set enrichment scores using GSVA. **D**. Heatmap of genes upregulated upon hypoxia and downregulated by onvansertib. **F**. Gene expression analysis by RT-qPCR in SW620 cells, data were normalized to *RPLP0* gene and 20%O_2-siCtrl sample.

6. Transcriptomic Changes Associated with Bevacizumab Treatment



RNA-seq of biopsies collected post treatment → Gene set enrichment analysis (GSEA) to identify pathways enriched in genes differentially expressed in Bev-exposed versus Bev-naïve patients

- Bev-exposed tumors were enriched for:
  - Several Cancer Hallmarks related to PLK1 functions, including hypoxia
  - Oncogenic signatures related to the angiogenic factors VEGF and PlGF
- These transcriptional changes may confer resistance to onvansertib and bevacizumab.



Conclusion

- In a Phase 1b/2 study for second-line treatment of *KRAS*-mutant mCRC patients, onvansertib + FOLFIRI/bevacizumab (Bev) showed superior clinical benefit in patients who did not receive Bev in first-line treatment (Bev-naïve) compared to patients who did.
- Based on these clinical data, the effect of onvansertib and Bev was investigated in *KRAS*-mutant CRC preclinical models:
  - *In vivo*, onvansertib + Bev resulted in potent antitumor activity in 3 xenograft models. Onvansertib reduced tumor vascularization and onvansertib + Bev resulted in greater decrease in tumor vascularization.
  - *In vitro*, onvansertib inhibited the activation of the hypoxia pathway through the regulation of the transcription factor HIF1α and its downstream targets.
- The clinical activity of onvansertib + chemotherapy/bevacizumab for the first-line treatment of *RAS*-mutated, Bev-naïve mCRC patients is currently being explored in a randomized study (NCT06106308).
  - See Poster #CT275 for more details.

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