OBI-992, a Novel TROP2 Antibody-Drug Conjugate, Exhibits Distinct Resistance Profiles Compared to other TROP2 Targeting ADCs

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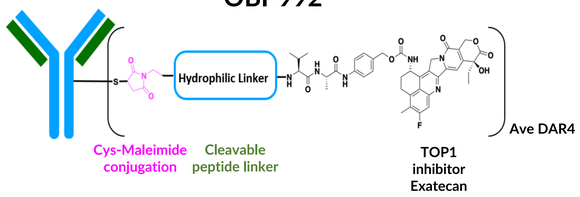
BACKGROUND

- Trophoblast cell surface antigen-2 (TROP2), a transmembrane glycoprotein overexpressed in various cancers, has been proposed as a potential cancer driver in multiple tumor types, including colorectal cancer (CRC)[1].
- TROP2 has emerged as a promising ADC target, with both sacituzumab govitecan (SG) and datopotamab deruxtecan (Dato-DXd) approved for the treatment of specific cancer types.
- One of the major challenges in ADC development is the emergence of resistance, while the underlying pathways remain poorly understood[2].
- OBI-992 is a novel TROP2-targeting ADC derived by conjugating a TROP2 antibody (R4702) with the TOP1 inhibitor exatecan. It is currently in Phase 1/2 clinical development[3].
- OBI-992 features a specially designed linker that enhances hydrophilicity and stability, distinguishing it from the unstable linker in SG and the high free payload exposure observed with Dato-DXd[4].



METHODS

- Anti-TROP2 ADC-resistant colorectal adenocarcinoma cell lines, DLD-1 were established in vitro using three anti-TROP2 ADCs including SG, Dato-DXd, and OBI-992. Cells were exposed to the ADCs for 3 days, followed by a recovery period. The treatment procedure involved stepwise increases in the concentrations of OBI-992 (100 nM to 2000 nM), SG (0.3 nM to 120 nM) and Dato-DXd (50 nM to 500nM) approximately 6 months. For OBI-992, an additional resistant subline with lower final concentration in 500 nM was generated.
- To evaluate the relative resistance of each TROP2 ADC–resistant cell line to its corresponding ADC (SG, Dato-DXd, and OBI-992) and payload (SN-38, Deruxtecan, and Exatecan), IC_{50} values were determined. DLD-1 cells were treated with serially diluted reagents for 6 days, and viability was assessed. IC_{50} values were calculated using Prism (GraphPad). Relative resistance was calculated as the ratio of IC_{50} between resistant and parental cells.
- To examine the protein levels of resistance-related proteins, western blot analyses were performed to detect TROP2, TOP1, and ABC transporters (BCRP and P-gp). To further confirm TROP2 surface expression, flow cytometry was conducted using two anti-TROP2 antibodies: a commercial antibody (BioLegend, #363803) and R4702, the antibody backbone of OBI-992.
- Whole Exome Sequencing (WES) and Variant Analysis. Resistant cells were extracted genomic DNA with the Human All Exon V8 probe set. Libraries were tagged with unique molecular identifiers (UMIs) to reduce PCR and sequencing errors. Paired-end sequencing (2×150 bp) was performed on an Illumina platform. Reads were aligned to the GRCh38/hg38 reference genome using the Illumina DRAGEN Bio-IT platform. Variant annotation was performed using the Ensembl Variant Effect Predictor (VEP).
- RNA-Seq and Transcriptomic Analysis. Total RNA was prepared using the Agilent SureSelect XT HS2 mRNA Library Preparation Kit. Sequencing was performed on the Illumina platform (2×150 bp). Reads were trimmed with Trimmomatic and aligned using HISAT2. Gene expression quantification was done with StringTie, and differential expression analysis was carried out using DESeq2. Genes with TPM < 0.1 were excluded. Differentially expressed genes (DEGs) were defined as those with fold change ≥ 2 and p-value ≤ 0.05.
- WES and RNA-seq analyses were conducted by Welgene Biotechnology Company (Taipei, Taiwan).

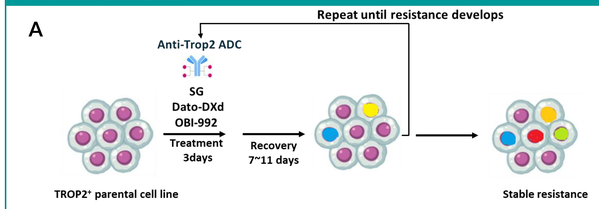
OBJECTIVE

To characterize resistance mechanisms of OBI-992 and compare them with those of approved TROP2 ADCs SG and Dato-DXd in CRC cells.

RESULTS

- TROP2-ADC–resistant cell lines were established by continuous exposure to SG, Dato-DXd, and OBI-992 until strong resistance to the corresponding ADCs was developed. For OBI-992, two resistant cell lines were generated at final concentrations of 0.5 µM and 2 µM, respectively. These cell lines also exhibited strong resistance to the payload. (Figure 1)

Figure 1. Establishment and Characterization of TROP2 ADC Resistant Cell Lines



B

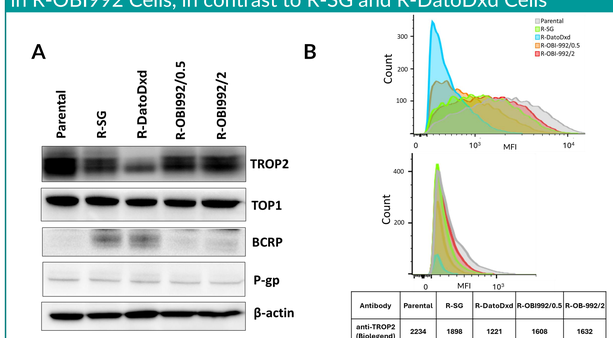
The relative resistance of TROP2-ADC resistant cell line to the corresponding ADC and payload

Resistant cell line	Corresponding ADC RR	Corresponding payload RR
1. R-SG	164	111
2. R-DatoDxd	>9[a]	19
3. R-OBI992/0.5	>9[a]	37
4. R-OBI992/2	>9[a]	48

(A) The scheme illustrates the establishment of TROP2 ADC–resistant cell lines. (B) The table shows the relative resistance (RR) of TROP2-ADC resistant cell line calculated by dividing the IC_{50} value of the resistant cells by that of the parental cells.

- TROP2 levels were significantly reduced in R-DatoDxd, while BCRP expression was upregulated in both R-SG and R-DatoDxd, but not detected in R-OBI992. Flow cytometry analysis further confirmed the reduction of surface TROP2 expression in R-DatoDxd. (Figure 2)

Figure 2. TROP2 Reduction and BCRP Induction were not Observed in R-OBI992 Cells, in contrast to R-SG and R-DatoDxd Cells

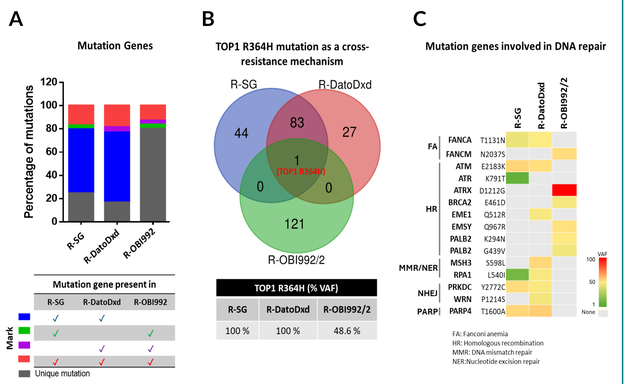


Antibody	Parental	R-SG	R-DatoDxd	R-OBI992/0.5	R-OB-992/2
anti-TROP2 (Biolegend)	2234	1898	1221	1608	1632
R4702	1615	1435	1022	1248	1320

(A) Western blot analysis in TROP2 ADC-resistant cells. (B) The surface protein level of TROP2 were detected by two anti-TROP2 antibody (Upper, Biolegend, #363803 , Down, R4702, the antibody backbone of OBI-992). Values are presented as geometric mean of MFI.

- WES analysis reveals that R-OBI992 possess a distinct genomic resistance profile, characterized by a lower allele frequency of the TOP1 inhibitor–resistant mutation TOP1 R364H—compared to 100% VAF observed in R-DatoDxd and R-SG and by unique mutations in DNA repair pathways, suggesting a resistance mechanism distinct from those of SG and Dato-DXd resistant cells.(Figure 3)

Figure 3. OBI-992–Resistant Cells Exhibit Low VAF of TOP1 R364H and a Resistance Profile Distinct from R-SG and R-DatoDxd Cells



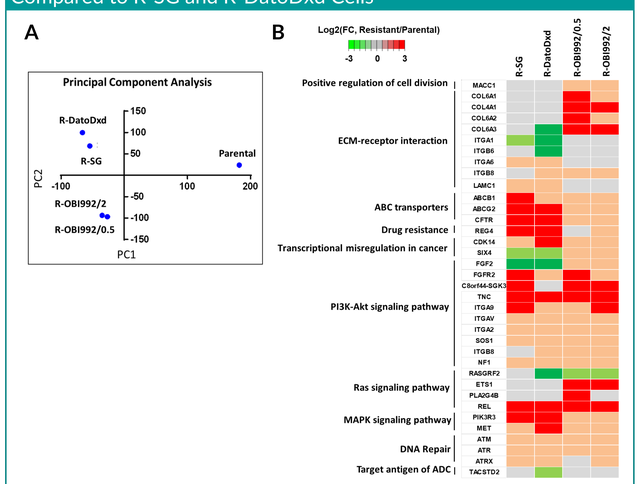
TOP1 R364H (% VAF)

R-SG	R-DatoDxd	R-OBI992/2
100 %	100 %	48.6 %

WES analysis of genetic mutations. (A) The oncogenic mutation genes and their overlap across resistant cells. (B) Venn diagram illustrates the overlap analysis of protein variant sites identified in each resistant cells. (C) Analysis of DNA repair-related mutations across resistant cells.

- RNA-seq revealed distinct clustering patterns among the resistant cell lines, with R-SG and R-DatoDxd clustering closely together, while R-OBI992 exhibited ECM-driven resistance, in contrast to the ABC transporter–linked resistance observed in R-SG and R-DatoDxd (Figure 4)

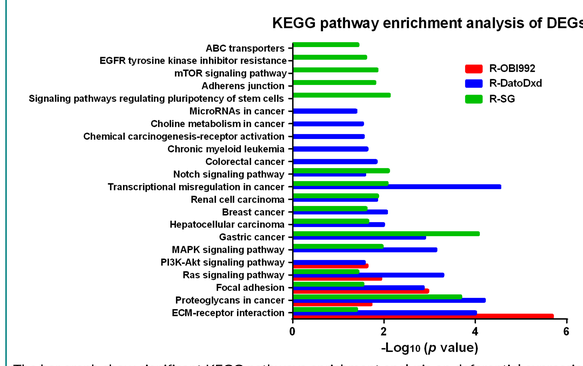
Figure 4. Distinct Transcriptomic Profile of R-OBI992 Cells Compared to R-SG and R-DatoDxd Cells



RNA-Seq analysis of TROP2-ADC-resistant Cells. (A) Principal Component Analysis (PCA) of transcriptomic profile.(B) Functional classification of altered transcriptional programs.

- KEGG pathway analysis of DEGs revealed prominent enrichment of ECM–receptor interaction in OBI-992–resistant cells, indicating a distinct resistance mechanism compared to SG and Dato-DXd. (Figure 5)

Figure 5. OBI-992-Resistant Cells Exhibit a Distinct ECM-Driven Transcriptomic Profile Compared to R-SG and R-DatoDxd Cells



The bar graph show significant KEGG pathways enrichment analysis on deferential expression genes (DEGs) across TROP2 ADC resistant cells.

CONCLUSIONS

- This study identifies resistance mechanisms against TROP2-targeting ADCs and highlights the distinct molecular resistance profile of OBI-992 compared to SG and Dato-DXd.
  - No evidence of TROP2 downregulation or BCRP upregulation was observed in R-OBI992 cells.
  - Genomic analysis revealed a lower VAF of the TOP1 inhibitor–resistant mutation R364H in R-OBI992, compared to 100% in R-SG and R-DatoDxd cells.
  - Transcriptomic profiling showed that OBI-992–resistant cells clustered separately from R-SG and R-DatoDxd cells.
  - KEGG analysis highlighted ECM-related signaling as a key adaptive pathway in OBI-992 resistance.
- This data suggests that OBI-992, a novel TROP2 ADC, emerges as a potential promising therapeutic option for patients who are resistant to SG and Dato-DXd, as it demonstrates a distinct resistant profile.

References
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Disclosures
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