Supplementary Material

Anti-NaPi2b antibody-drug conjugate lifastuzumab vedotin (DNIB0600A) compared to pegylated liposomal doxorubicin in patients with platinum-resistant ovarian cancer in a randomized, open-label, phase II study


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Selection of patients, including both eligibility and ineligibility criteria

PATIENT SELECTION

Inclusion Criteria

1. Signed informed consent form
2. Age $\geq 18$ years
3. Life expectancy of at least 12 weeks
4. Eastern Cooperative Oncology Group (ECOG) Performance Status of 0 or 1
5. Histological documentation of epithelial ovarian cancer, primary peritoneal cancer, or fallopian tube cancer (excluding carcinosarcoma histology)
6. Availability and willingness to provide an adequate archival sample of tumor (paraffin tissue block or at least 15 unstained slides); if an archival tissue specimen is not available and a new tissue specimen is collected for diagnostic purposes for patient care, then fresh tissue may be submitted
7. Advanced epithelial ovarian, primary peritoneal, or fallopian tube cancer that has progressed or relapsed during or within 6 months after the most recent treatment with a platinum-containing chemotherapy regimen and for whom PLD is appropriate therapy. Progression or relapse from prior platinum-based chemotherapy must be documented radiographically by RECIST v1.1 criteria.
8. Measurable disease with at least one lesion that can be accurately measured in at least one dimension (longest dimension recorded $\geq 2.0$ cm using conventional techniques or $\geq 1.0$ cm on spiral computed tomography [CT] scan) per RECIST v1.1 criteria
9. No more than one prior cytotoxic chemotherapy regimen for the treatment of platinum-resistant ovarian cancer (PROC) and no more than two total regimens (defined as any therapy [approved or investigational] with intent to treat the ovarian cancer)
10. Absolute neutrophil count $\geq 1500/\mu$L, hemoglobin $\geq 9$ g/dL, and platelet count $\geq 100,000/\mu$L
11. Total bilirubin $\leq 1.5 \times$ upper limit of normal (ULN); aspartate aminotransferase (AST) and alanine aminotransferase (ALT) $\leq 2.5 \times$ ULN
12. Serum creatinine $\leq 2.0$ mg/dL
13. International normalized ratio (INR) $\leq 1.5$ and activated partial thromboplastin time (aPTT) $\leq 1.5 \times$ ULN in the absence of anticoagulation therapy. If patients are on anticoagulation therapy, INR should be within the therapeutic range for the medical indication.
14. Willing and able to perform a PRO survey (including the possibility of using an electronic PRO [ePRO] device)
15. For women of childbearing potential, agreement to use one highly effective form of non-hormonal contraception or one highly effective form and one effective form of non-hormonal contraception through the course of study treatment and for 6 months after the last dose of study treatment

- A woman is considered not to be of childbearing potential if she is post-menopausal, defined by amenorrhea for ≥ 12 months and age ≥ 45 years, or has undergone hysterectomy and/or bilateral oophorectomy

- The following are considered highly effective forms of contraception: 1) true abstinence and 2) male sterilization (with post procedure documentation of absence of sperm in the ejaculate); the sterilized male partner should be the sole partner

- The following are considered effective forms of contraception: 1) intrauterine device (IUD) or intrauterine system (IUS); 2) condom with spermicidal foam/gel/film/cream/suppository; and 3) occlusive cap (diaphragm or cervical/vault cap) with spermicidal foam/gel/film/cream/suppository

16. For women of childbearing potential, a negative serum pregnancy test within 72 hours prior to commencement of dosing; women who are considered not to be of childbearing potential are not required to have a pregnancy test

**Exclusion Criteria**

1. Primary platinum-refractory disease defined as disease progression during or within 2 months of a first-line, platinum containing chemotherapy regimen

2. Anti-tumor therapy, including chemotherapy, biologic, experimental, or hormonal therapy, within 4 weeks prior to Day 1

3. Palliative radiation within 2 weeks prior to Day 1

4. Prior anthracycline therapy, including prior treatment with PLD (e.g., Doxil®, Caelyx®, or Lipodox®) in any setting (e.g., in combination with carboplatin or as a single agent)

5. Prior treatment with NaPi2b or SCL34A2 targeted therapy

6. Major surgical procedure within 4 weeks prior to Day 1

7. Current Grade > 1 toxicity (except alopecia and anorexia) from prior therapy or Grade > 1 neuropathy from any cause

8. Left ventricular ejection fraction (LVEF) defined by multigated acquisition (MUGA) or echocardiogram below the institutional lower limit of normal (LLN)

9. Evidence of significant, uncontrolled, concomitant disease that could affect compliance with the protocol or interpretation of results, including significant cardiovascular disease (such as New York Heart Association Class III or IV cardiac disease, congestive heart failure, myocardial infarction within the last 6 months, unstable arrhythmias, or unstable angina) or significant pulmonary disease
(including obstructive pulmonary disease, history of bronchospasm, or any ongoing requirement for supplemental oxygen)

10. Known active bacterial, viral, fungal, mycobacterial, parasitic, or other infection (including HIV and atypical mycobacterial disease but excluding fungal infections of the nail beds) at study enrollment or any major episode of infection requiring treatment with IV antibiotics or hospitalization (relating to the completion of the course of antibiotics) within 4 weeks prior to Cycle 1, Day 1

11. Clinically significant history of liver disease, including viral or other hepatitis, current alcohol abuse, or cirrhosis

12. Presence of positive test results for hepatitis B (HBsAg and/or total hepatitis B core antibody [anti HBc]) or hepatitis C (HCV antibody)
   a. Patients who are positive for anti HBc are eligible only if the polymerase chain reaction (PCR) testing is negative for hepatitis B virus (HBV) DNA and it is believed by both the investigator and Medical Monitor to be in the patient’s best interest to participate
   b. Patients who are positive for HCV antibody must be negative by PCR to be eligible for study participation

13. Known history of HIV seropositive status

14. Other malignancy within the last 5 years, except for adequately treated carcinoma in situ of the cervix, squamous carcinoma of the skin, adequately controlled limited basal cell skin cancer, or synchronous primary endometrial cancer or prior primary endometrial cancer if all of the following criteria are met:
   a. Stage ≤ IB
   b. Superficial myometrial invasion without vascular or lymphatic invasion
   c. No poorly differentiated subtypes (i.e., papillary serous, clear cell, or other Federation of Gynecology and Obstetrics [FIGO] Grade 3 lesions)

15. Untreated or active central nervous system metastases (progressing or requiring anticonvulsants or corticosteroids for symptomatic control)

16. Pregnancy or breastfeeding

17. Known history of NaPi2b deficiency (e.g., congenital alveolar microlithiasis or testicular microlithiasis)

18. History of severe allergic or anaphylactic reactions to monoclonal antibody therapy (or recombinant antibody related fusion proteins)

19. Any other diseases, metabolic dysfunction, physical examination finding, or clinical laboratory finding giving reasonable suspicion of a disease or condition that contraindicates the use of an investigational drug or that may affect the interpretation of the results or render the patient at high risk from treatment complications
Biomarker assessments

For determination of NaPi2b protein expression in formalin-fixed, paraffin-embedded archival tissues, a fully automated IHC assay was developed using the anti-NaPi2b (10H1) mouse monoclonal antibody (Genentech, Inc., South San Francisco, CA) and ultraView DAB IHC Detection (Ventana Medical Systems, Inc., Tucson, AZ). NaPi2b membranous staining level was scored according to the following algorithm, where at least 50% of tumor cells had to be stained in order to qualify as positive in each category; IHC=3+, the predominant staining intensity was 3+ denoting predominantly strong staining; IHC=2+, predominantly moderate staining; IHC=1+, predominantly weak staining; IHC=0, very weak or no staining in >50% of tumor cells. NaPi2b H score was defined as \[1^* (% \text{ cells staining at IHC} 1+) + 2^* (% \text{ cells staining at IHC} 2+) + 3^* (% \text{ cells staining at IHC} 3+)\]. NaPi2b transcript levels in the tumor tissues were also determined by qRT-PCR using a validated NaPi2b/TMEM (house-keeping gene) duplex assay (Cobas z480 Real-time PCR Platform), (Roche Molecular Systems, Pleasanton, CA).
Statistical analyses

Assuming 3.7 months of median PFS time in the PLD arm and approximately 85% of patients to have NaPi2b-high tumors by IHC, 92 patients were required to achieve 80% power to detect a hazard ratio (HR) of 0.45 at 2-sided significance level of 5%. Primary (PFS) and secondary (OR, OS) efficacy analyses included all 95 randomized patients in the intent-to-treat (ITT) population. While all patients were followed to progression, the PFS primary endpoint was analyzed when approximately 59 investigator-assessed PFS events occurred (defined as either disease progression per RECIST v1.1 or death, whichever occurred first). At the time of data cutoff, approximately 50 PFS events were anticipated among NaPi2b-high patients; NaPi2b-high patients were pre-defined as IHC 2+/3+ with a 50% cutoff for staining. Kaplan Meier methods were used to estimate the median PFS for each treatment arm. Safety analyses included all randomized patients in the two treatment arms who received any amount of treatment.
Supplementary table and figures

**Supplementary Table S1. Exposure and Pharmacokinetics of Analytes**

<table>
<thead>
<tr>
<th></th>
<th>$C_{\text{max}}$ (ng/ml)</th>
<th>$\text{AUC}_{\text{inf}}$ (day*ng/ml)</th>
<th>$t_{1/2}$ (day)</th>
<th>$V_{\text{ss}}$ (ml/kg)</th>
<th>$\text{CL}$ (ml/kg/day)</th>
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<tbody>
<tr>
<td>acMMAE (n=41)</td>
<td>855 (166)</td>
<td>2685 (611)</td>
<td>5.1 (1.2)</td>
<td>70 (19)</td>
<td>16 (3.7)</td>
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<tr>
<td>Total antibody (n=41)</td>
<td>47000 (10000)</td>
<td>243000 (58000)</td>
<td>7.0 (1.9)</td>
<td>76 (22)</td>
<td>10 (2.7)</td>
</tr>
<tr>
<td>Unconjugated MMAE (n=41)</td>
<td>3 (2.4)</td>
<td>27.4 (19.6)</td>
<td>6.7 (2.2)</td>
<td>-</td>
<td>-</td>
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</tbody>
</table>
Platinum-resistant ovarian cancer N=95 all-comers

Randomized n = 95

Lifastuzumab (LIFA)
2.4 mg/kg IV Q3W
(n = 47)
Treat to progressive disease

Received drug (n = 46)
Discontinued drug (n = 33)
- death (n = 0)
- adverse event (n = 4)
- patient decision (n = 0)
- physician decision (n = 0)
- disease progression (n = 29)

Survival analysis
- analyzed (n = 47)
- excluded (n = 0)

PK analysis
- analyzed (n = 41)
- excluded (n = 6)

Pegylated liposomal doxorubicin (PLD)
40 mg/m² IV Q4W
(n = 48)
Treat to progressive disease

Received drug (n = 47)
Discontinued drug (n = 38)
- death (n = 0)
- adverse event (n = 4)
- patient decision (n = 1)
- physician decision (n = 2)
- disease progression (n = 31)

Survival analysis
- analyzed (n = 48)
- excluded (n = 0)

Supplementary Figure S1. CONSORT diagram.
**Supplementary Figure S2.** Investigator-assessed best radiographic response. NaPi2b-high and NaPi2b-low patients were defined by immunohistochemistry assessments of NaPi2b expression.

(A) Lifestuzumab vedotin arm (N=47)

(B) Pegylated liposomal doxorubicin arm (N=48)
**Supplementary Figures S3. Efficacy subgroup analysis by biomarker subsets.**

**Progression free survival**

- **IHC 3+ subgroup**
  - Median survival:
    - **LIFA**: 5.3 months
    - **PLD**: 3.3 months
  - Hazard ratio: 0.66
  - 95% CI (0.35, 1.27)
  - *p* = 0.213

- **H-score high subgroup**
  - Median survival:
    - **LIFA**: 5.5 months
    - **PLD**: 2.9 months
  - Hazard ratio: 0.47
  - 95% CI (0.19, 1.21)
  - *p* = 0.109

- **qPCR high subgroup**
  - Median survival:
    - **LIFA**: 5.5 months
    - **PLD**: 3.2 months
  - Hazard ratio: 0.54
  - 95% CI (0.22, 1.31)
  - *p* = 0.174

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<table>
<thead>
<tr>
<th>Group</th>
<th>ITT (n=95)</th>
<th>NaPi2b 2/3+ (n=85)</th>
<th>NaPi2b 3+ (n=70)</th>
<th>NaPi2b median H-score high (n=43)</th>
<th>NaPi2b median qPCR high (n=41)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arm</td>
<td>LIFA n=47</td>
<td>PLD n=48</td>
<td>LIFA n=42</td>
<td>PLD n=43</td>
<td>LIFA n=31</td>
</tr>
<tr>
<td>ORR</td>
<td>34%</td>
<td>15%</td>
<td>36%</td>
<td>14%</td>
<td>42%</td>
</tr>
<tr>
<td>Median PFS (months)</td>
<td>5.3</td>
<td>3.1</td>
<td>5.3</td>
<td>3.4</td>
<td>5.3</td>
</tr>
<tr>
<td>HR</td>
<td>0.78 (0.34)</td>
<td>0.71 (0.24)</td>
<td>0.66 (0.21)</td>
<td>0.47 (0.11)</td>
<td>0.54 (0.0174)</td>
</tr>
</tbody>
</table>
Supplementary Figure S4. Maximal CA-125 decreases in (A) the LIFA arm, and (B) the PLD arm.
Supplementary Figure S5. Maximal HE4 decreases in (A) the LIFA arm, and (B) the PLD arm.