Preliminary Results from PiSARRO, a Phase Ib/II Study of APR-246, a Mutant p53 Reactivating Small Molecule, in Combination with Standard Chemotherapy in Platinum Sensitive Ovarian Cancer

Abstract #CT204

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PiSARRO, p53 Suppressor Activation in Recurrent High Grade Serous Ovarian Cancer, is a recently commenced Aprea/EUTROC phase Ib/II study of APR-246 in combination with carboplatin and pegylated liposomal doxorubicin (PLD) in recurrent p53 mutant platinum sensitive high grade serous ovarian cancer.

Introduction:

- p53 is a key tumor suppressor that induces cell cycle arrest, senescence and/or apoptosis upon cellular stress, eliminating tumor cells. p53 mutations are found in more than 50% of cancers and are associated with increased resistance to chemotherapy.
- Mutations in p53 correlate with chemotherapy resistance, early relapse and shortened overall survival (1).
- Despite high response rates from carboplatin in combination with paclitaxel in first-line treatment of ovarian cancer, most patients relapse and develop resistance.
- In High Grade Serous Ovarian Cancer (HGSOC) 96% of patients have p53 mutations (2).

Background:

- APR-246 (PRIMA-1^{MET}) is a pro-drug that is converted to the active form MQ, which restores mutant p53 to the wild type conformation (3).
- APR-246 is the first clinical-stage compound that reactivates mutant p53.
- In the first-in-human Phase Ia study, APR-246 monotherapy was found to have a satisfactory safety and pharmacokinetic profile allowing it to be combined with full dose chemotherapy. Signs of single agent clinical activity were observed in several patients, and p53-dependent biological effects in patient tumor cells were demonstrated (5). APR-246 was considered safe in patients, with infrequent fully reversible CNS related side effects (dizziness, dyskinesia and ataxia). No bone marrow toxicity was seen (4).
- APR-246 has been shown in vitro to reduce glutathione levels, increase ROS levels and ER stress, and to resensitize ovarian cancer cells to platinum drugs (5, 6).
- APR-246 displays strong synergy with conventional chemotherapeutic drugs in primary ovarian cancer cells *ex vivo* (Fig. 1) (5, see also 7).
- APR-246 reactivates p53 and resensitizes tumor cells to cisplatin, forming a strong rationale for combination treatment with APR-246 and platinum-based chemotherapy.

Clinical study design:

- The ongoing Phase Ib/II study is enrolling patients with recurrent partially platinum sensitive (PFI 6-12 mo) and platinum sensitive (PFI 12-24 mo) HGSOC with positive p53 staining on immunohistochemistry.
- APR-246 is administered as a 6h i.v. infusion on 4 consecutive days every 4 weeks for 6 cycles. On day 4, APR-246 is given concomitantly with carboplatin AUC 5 and PLD 30 mg/m2.
- The Phase Ib part has a 3+3 dose escalation design with 3 planned dose levels (Fig. 2) based on safety evaluation after cycle one for dose escalation.
- Phase II dose selection will be based on short and long term safety as well as preliminary efficacy data.
- In the Phase II part, 164 patients with up to two prior lines of platinum based therapy with a PFI of 6-24 months with measurable disease available for pre- and on treatment biopsies will be randomized to standard chemotherapy with or without APR-246.
- Patients are followed for safety, response (Recist 1.1 and CA125 (GCIC criteria)), progression and survival as well as several exploratory endpoints.

Translational studies:

- A comprehensive exploratory translational science program with repeat tumor biopsies is included in the study as part of the EUTROC investigators group. The key objectives are to identify potential biomarkers for patient selection and for monitoring response to treatment and to further our understanding of the MOA.
- p53 will be sequenced in tumor biopsies. Mutations will be classified structurally, and the possible correlation of treatment response with mutations and/or type of mutant p53 structure will be assessed.
- p53 will also be sequenced from circulating free tumor DNA.
- Circulating cytokeratin 18 in serum will be measured by ELISA, to follow epithelial cell death and apoptosis.
- ER stress biomarkers will be analyzed in tumor biopsies using IHC.
- Multiple markers will be studied using reverse phase protein array and mRNA microarray analyses.

Preliminary results:

- To date patients have been enrolled to all 3 dose cohorts of the Phase Ib and the patients in the first cohort have completed therapy. In the first dose cohort 2 patients were partially platinum sensitive and 1 sensitive.
- One DLT of ruptured diverticulum occurred at the 2nd dose level leading to expansion of this cohort to 6 patients.
- Main AEs have been hematological (neutropenia, thrombocytopenia), and low grade CNS related effects (dizziness, vertigo, nausea, dysgeusia). No new safety concerns have emerged (Table 1).

Table 1: Summary of Treatment-Emergent Adverse Events by System Organ Class

Safety-Evaluable (N=15)

MedDRA System Organ Class [a][b]	All TEAEs	Related Any Grade[c]	Any Relationship >=Grade 3[c]	Related >=Grade 3[c]
Number of Patients	15	15	15	15
Patients with Any TEAEs	13 (86.7%)	13 (86.7%)	9 (60.0%)	5 (33.3%)
Nervous system disorders	12 (80.0%)	10 (66.7%)	0	0
Blood and lymphatic system disorders	11 (73.3%)	6 (40.0%)	7 (46.7%)	4 (26.7%)
General disorders and administration site conditions	11 (73.3%)	10 (66.7%)	0	0
Gastrointestinal disorders	10 (66.7%)	9 (60.0%)	2 (13.3%)	1 (6.7%)
Infections and infestations	8 (53.3%)	1 (6.7%)	4 (26.7%)	0
Musculoskeletal and connective tissue disorders	5 (33.3%)	1 (6.7%)	0	0
Respiratory, thoracic and mediastinal disorders	4 (26.7%)	0	0	0
Injury, poisoning and procedural complications	3 (20.0%)	0	0	0
Metabolism and nutrition disorders	3 (20.0%)	2 (13.3%)	1 (6.7%)	0
Psychiatric disorders	2 (13.3%)	0	0	0
Skin and subcutaneous tissue disorders	2 (13.3%)	0	0	0
Cardiac disorders	1 (6.7%)	0	0	0
Immune system disorders	1 (6.7%)	0	0	0
Investigations	1 (6.7%)	1 (6.7%)	1 (6.7%)	0
Renal and urinary disorders	1 (6.7%)	0	0	0
Reproductive system and breast disorders	1 (6.7%)	0	0	0
Vascular disorders	1 (6.7%)	1 (6.7%)	0	0

[a] Number of Patients used as denominator to calculate percentages.

[b] Patients with multiple TEAEs were counted once within a summary category: system organ class, preferred term, maximum grade, or relationship to treatment. Patients with events in more than one category were counted once within each category. Treatment-Emergent Adverse Events (TEAEs) were defined as all AEs that occurred after the first dose of study medication or within 30 day post-treatment period.

[c] Grade: 1=Mild, 2=Moderate, 3=Severe, 4=Life-Threatening, 5=Fatal.

- APR-246 showed linear pharmacokinetics with no accumulation and low inter- and intra- patient variability (Fig. 3). No indication of interaction between APR-246 and chemotherapy was seen.
- The first 3 patients have completed 6 cycles of therapy and are now in follow up. All 3 had partial response (PR) by RECIST and the 2 patients evaluable for CA125 response showed response according to GCIG (Fig. 4) with normalization of their CA125.

Fig. 1: Synergistic effect of APR-246 with cisplatin on primary cancer cells from 5/5 examined ovarian cancer patients.

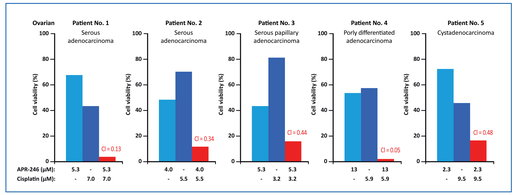


Fig. 2: PiSARRO – a Phase Ib/II study in platinum sensitive p53 mutant High Grade Serous Ovarian Cancer.

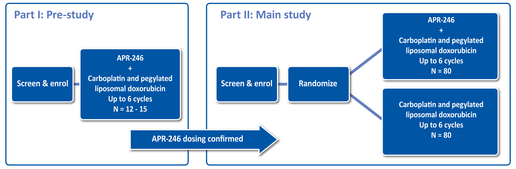


Fig. 3: APR-246 showed linear pharmacokinetics with no accumulation and low inter- and intra- patient variability. No indication of interaction between APR-246 and chemotherapy was seen.

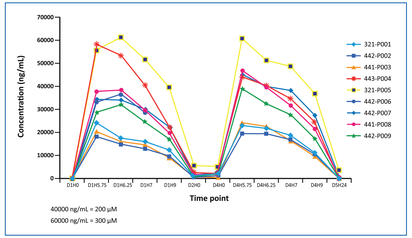
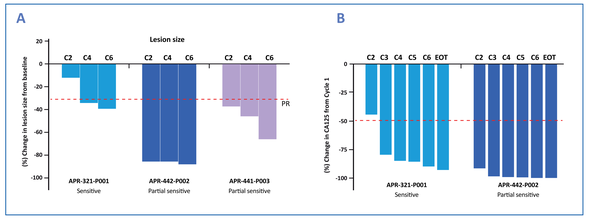


Fig. 4: The first 3 patients have completed 6 cycles of therapy and are now in follow up. All 3 had partial response (PR) by RECIST (A) and the 2 patients evaluable for CA125 response showed response according to GCIG with normalization of their CA125 (B).



Conclusions:

- Preliminary data from the PiSARRO phase Ib study indicate that APR-246 can be combined with carboplatin and PLD at relevant doses.
- A possible increase of the chemotherapy related hematological side effects cannot be ruled out at this stage.
- The preliminary efficacy data indicate that APR-246 in combination with chemo-therapy has activity in patients with PFI 6-12 mo as well as with PFI 12-24 mo.
- APR-246 in combination with chemotherapy has an encouraging safety and activity profile, supporting continuation of the study in Phase II as soon as the recommended dose for Phase II has been established.

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Disclosures

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Strong Synergy with APR-246 and DNA-Damaging Drugs in Primary Ovarian Cancer Cells

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Abstract #1639

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Introduction:

- Mutations in the tumor suppressor gene *TP53* occur in at least 60% of ovarian tumors and are associated with chemoresistance and poor prognosis. High-grade serous (HGS) ovarian cancer, which is the most common type of ovarian cancer, has the highest frequency of *TP53* mutations (96.7%) of any solid cancer (1).
- APR-246 (PRIMA-1^{MET}) is the first mutant p53-protein reactivating compound in clinical development. It has been tested as monotherapy in a Phase I/IIa clinical trial with hematological malignancies and prostate cancer with encouraging results (2). A Phase Ib/II study with APR-246 in combination with carboplatin and pegylated liposomal doxorubicin (PLD) in p53-mutant ovarian cancer is ongoing (3).
- APR-246 belongs to a class of small molecules, quinuclidinones. It is a prodrug that is converted to the active form MQ, a Michael acceptor that binds to mutant and/or unfolded p53 and restores correct wild type conformation, thus triggering apoptosis (4).
- We have previously demonstrated synergistic anticancer effect with APR-246 and cisplatin in ovarian cancer cell lines with various p53 status. The aim of this study was to investigate the anticancer effect of APR-246 as single compound as well as in combination with the DNA-damaging drugs cisplatin, carboplatin and doxorubicin in primary cancer cells isolated from ascites fluid from ovarian cancer patients.

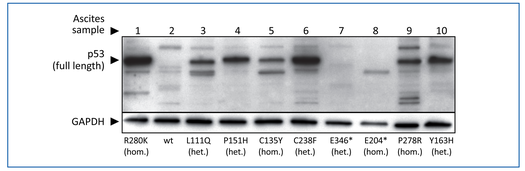
Methods:

- Written informed concent was obtained before ascites fluid was collected, and the procedures were approved by ethics committee and were in accordance with the principles of the Declaration of Helsinki.
- Eight of ten samples tested were from patients with recurrent ovarian cancer previously treated with platinum drugs. Six samples were poorly differentiated serous carcinoma and four were medium to well differentiated.
- Cancer cells were purified by Ficoll and viably frozen. The quality and purity was confirmed by May Grünwald/Giemsa staining. For some samples, immunocytochemical staining with anti-Ber-EP4 antibody and anti-calretinin antibody was needed in order to distinguish between mesothelial and cancer cells. Anti-Ber-EP4 antibody stains all cells of epithelial origin including ovarian cancer cells, whereas anti-calretinin antibody stains mesothelial cells. Secondary staining was performed with Dako REAL EnVision Detection System and visualized with diaminobenzidine (DAB) according to the kit.
- According to quality criteria at least 70% of all cells in a sample should be cancer cells and the cell viability should be at least 70%. All samples included in the study met these criteria as visually judged by an experienced cytopathologist.
- Cell viability was assessed with the fluorometric microculture cytotoxicity assay (FMCA) (5). Combination Index (CI) was calculated using Additive model (6); CI = 1.0 ± 0.2 indicates additive effect, CI < 0.8 indicates synergy and CI < 0.5 indicates strong synergy.
- *TP53* gene status was determined by Sanger sequencing and single strand conformation analysis, and p53 protein expression by Western blotting.

Results and Discussion:

- Cancer cells from seven patients had relatively rare *TP53* core domain missense mutations (L111Q, C135Y, P151H, Y163H, C238F, P278R and R280K), two had stop codons (E346* and E204*), and one had wild type p53. All of the missense mutations are in the DNA-binding domain and have been predicted to severely affect the p53 tumor suppressor function (http://p53.free.fr). Ovarian cancer is characterized by a high frequency of frame shift mutations (15% of p53-mutant tumors).
- Missense mutant p53 proteins were expressed at high levels, while no p53 expression was detected in cells carrying nonsense mutant p53 or wild type p53 (Fig. 1).

Figure 1. p53 protein expression. The polyclonal anti-p53 antibody (#9282, Cell Signaling) binds strongly to the N-terminal of the p53 protein, and more weakly also to the DNA-binding region of p53.



* = stop codon

- Strong synergistic effects of APR-246 and cisplatin in cells with homozygous R280K p53 mutation were observed (Fig. 2).
- Table 1 summarizes the results from combination studies with APR-246 and cisplatin in all samples, together with diagnosis and histological description, prior chemotherapy and p53 status. Synergistic or strong synergistic effects with cisplatin were shown in all samples tested.
- The IC_{50} value of APR-246 ranged from 5 to 56 µM and the IC_{50} value of cisplatin from 3 to 45 µM in cancer cells from various patients.
- Strong synergy was also observed with the platinum analog carboplatin (Fig. 3A) and the anthracyclin doxorubicin (Fig. 3B) in cancer cells from a patient carrying homozygous C135Y p53 mutation.
- We also investigated the ability of APR-246 to sensitize the primary ovarian cancer cells to cisplatin using cancer cells carrying homozygous P278R mutant p53 from a patient who had received chemotherapy. As shown in Fig. 4, APR-246 increased the sensitivity of the cells to cisplatin in a dose-dependent manner. The IC50 value decreased from 9.2 to 2.3 µM in the presence of 8 µM APR-246.

Figure 2. Strong synergistic effects with APR-246 and cisplatin in primary cancer cells from patients with p53 mutant ovarian cancer. Results are mean±SEM (n=2).

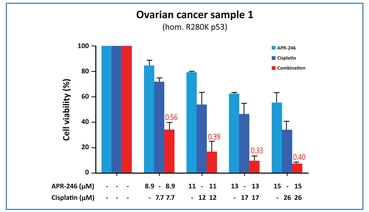


Table 1. Summary of combination effects with APR-246 and cisplatin in primary ovarian cancer cells. Synergistic (CI <0.8) or strong synergistic effects (CI <0.5) with APR-246 and cisplatin were observed in all samples. n=2 (except for sample 6 and 7 where n=1).

Sample No.	Diagnosis[§]	Histological description[^]	Prior Chemo.	p53 status	Combination APR-246 cisplatin
1	Ovarian cancer stage III	Poorly differentiated serous carcinoma[#]	Yes	Hom. R280K	SS
2	Cancer peritonei stage IV	Serous carcinoma	Yes	wt	S, Add
3	Ovarian cancer stage IC	Serous carcinoma	Yes	Het. L111Q	S
4	Ovarian cancer/Cancer peritonei stage IV	Poorly differentiated serous carcinoma[#]	Yes	Het. P151H	SS
5	Cancer peritonei stage IIIC	Serous adenocarcinoma, grade III[#]	No	Hom. C135Y	SS
6	Tubar cancer stage IIIB	Poorly differentiated serous adencarcinoma[#]	Yes	Het. C238F	SS
7	Ovarian cancer stage IIIC	Poorly differentiated serous carcinoma[#]	Yes	Het. E346*	S, Add[^^]
8	Cancer peritonei IV	Medium-well differentiated serous carcinoma	No	Hom. E204*	SS
9	Ovarian cancer stage IIIC	Poorly differentiated serous carcinoma[#]	Yes	Hom. P278R	SS
10	Ovarian cancer stage IIIC	Medium-well differentiated serous adencarcinoma	Yes	Het. Y163H	SS

Het. = heterozygous
Hom. = homozygous
* = stop codon

Add = additive (Combination index = 1.0 ± 0.2)
S = synergy (Combination index < 0.8)
SS = strong synergy (Combination index < 0.5)
Prior chemo. = The patient had been treated with chemotherapy.

§ The ten samples included in the study were from patients diagnosed with ovarian, peritoneal or fallopian tube cancer. Accumulated evidence suggest that high-grade serous carcinomas (HGSC) found in these tissues share a similar origin and pathogenesis pointing out the distal part of the fallopian tubes as the origin in the majority of cases (7, 8).

^ The primary histopathological analysis of the samples in this study was done using the previous grading system and heterogenous criteria and not according to the present standard where tumors are classified into low- and high-grade serous carcinomas.

These samples have poorly differentiated cells and are HGSC. Likely also the other samples with p53 mutation are HGSC.

¤ The sequencing method used cannot distinguish homozygous from hemizygous mutations, neither can heterozygosity be distinguished from a mixture of cells with wt p53 and cells with mutant p53.

^^ Cisplatin and APR-246 had high IC_{50} values in sample 7 and the results were variable.

Figure 3. Strong synergistic effects with APR-246 and carboplatin (A) or doxorubicin (B) in primary cancer cells from patients with p53 mutant ovarian cancer. Results are mean±SEM (n=2).

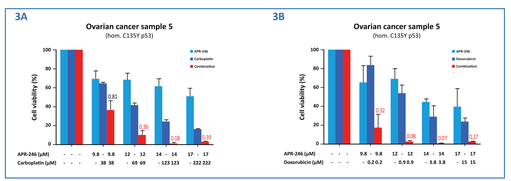
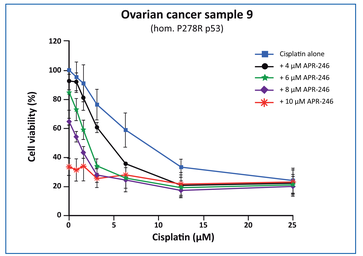


Figure 4. APR-246 sensitizes primary ovarian cancer cells to cisplatin. The IC_{50} value decreased from 9.2 to 2.3 µM in the presence of 8 µM APR-246. Results are mean±SEM (n=2).



Conclusions:

- We observed synergistic or strong synergistic effects of APR-246 and the DNA-damaging drugs cisplatin, carboplatin and doxorubicin in primary ovarian cancer cells with various p53 status.
- Our results provide a strong rationale for the ongoing clinical study with APR-246 in combination with carboplatin and PLD in patients with ovarian cancer (3), and suggest that combination treatment with APR-246 and DNA-damaging drugs could allow significantly improved treatment for ovarian cancer carrying mutant p53.

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Disclosures

Fransson, Alfredsson, Mohell: Aprea AB: Employment. Wiman: Aprea AB: Co-founder, shareholder and member of the board.