Toca 511-mediated Pro-drug Activator Gene Therapy: A Promising Therapeutic Strategy for Ovarian Cancer

Sara A. Collins¹, Priyanka Kamath¹,2 , Suzanne Matsuura¹, Douglas J. Jolly³, Brian Slomovitz¹,2, Noriyuki Kasahara¹,4

¹Sylvester Comprehensive Cancer Center, ²Department of Gynecologic Oncology, ³Tocagen Inc., San Diego, CA, USA, ⁴Department of Pathology, University of Miami, Miami, FL, USA

Introduction
Ovarian cancer is the leading cause of death from gynecologic malignancies in the U.S. and the fourth leading cause of cancer deaths in women. The disease is under investigation in multi-center ascending dose trials in patients with recurrent high grade glioma (http://www.clinicaltrials.gov; NCT01156584, NCT01985256, NCT01470794), and on based highly encouraging evidence of therapeutic benefit, a registrational Phase III trial has been initiated at multiple sites in the United States, Canada and South Korea (NCT02414165). Following completion of a phase 1 dose ascending trial for intravenous delivery of RRV (NCT01985256), it is timely to consider this approach also to systemic malignancies such as ovarian cancer. Accordingly, we have conducted the first preclinical studies to evaluate feasibility, safety and efficacy of RRV-mediated prodrug activator gene therapy for ovarian cancer.

Methods and Results

1. RRV Vectors used in this study

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2. Cell Lines used in this study

Established human ovarian cancer cell lines:  
- SKOV3-IP – human ovarian carcinoma  
- Ovca5 – human ovarian epithelial carcinoma  
- ES2 – human ovarian clear cell carcinoma

Patient Derived Primary Human Isolates:  
- C5x – clear cell carcinoma
- PSX – papillary serous

Transgenic model-derived primary murine ovarian cancer cells:  
- Mosep53mut – Mouse ovarian surface epithelium from a p53 floxed mouse, Ad-CRE to generate p53 mutant in vitro
- Mosep53KO – Mouse ovarian surface epithelium from a C57/B6 transgenic p53KO mouse

3. In vitro replication kinetics of RRV-EMD in Ovarian Cancer Cells

A. RRV Kinetics in Primary Ovarian Cells

B. RRV Kinetics in Commercial Ovarian Cancer Cell lines

Various ovarian cell lines were transduced with RRV-EMD at MOI = 0.01 and analyzed for GFP expression by flow cytometry every 3rd day after virus administration. RRV spread in (A) Patient derived primary ovarian cancer isolates, (B) Ovarian cancer cell lines and (C) established human ovarian cancer cell lines. Demonstrated efficient RRV spread in both primary and established ovarian cancer cell lines.

4. In vitro cytotoxicity assay in 100 % transduced Ovarian Cancer Cells

5-FC-mediated killing of transduced ovarian cancer cells. Cells fully transduced with the RRV-EMD or RRV-CD (Toca 511) vectors were seeded at a density of 1 x 10⁶ cells/well. 24 hr post-seeding, cells were exposed to 5-FC at 0 mM, 0.001 mM, 0.01 mM, 0.1 mM, 1 mM or 10 mM concentrations for 24 hr. MTS assay was carried out 3, 6 and 9 days post 5-FC exposure.

Significant cytotoxicity was induced in RRV-CD transduced ovarian cancer cells at 5-FC concentrations > 0.1 mM.

6. Optimization of vector delivery – Dose escalation

10-fold increase in RRV dose improves efficiency of gene delivery in vivo

Vectors administered IP using two regimens:  
- Multiple low dose (4 x 10⁵ TU/mouse/day in 0.5 ml x 4 days) or Single high dose (2 x 10⁶ TU/mouse in 1 ml)

On days 16, 23 and 36 post vector administration mice were euthanized and tumor cells were isolated from the omentum/mesentery and analyzed as described above. Isolation of human cells was confirmed as indicated by the shift along the red axis when cells were stained with the HLA antibody. GFP+ cells were detected on day 17 post vector administration with an increase in the number of GFP+ cells observed on days 23 and 36. No difference was observed when the virus was administered either as a bolus or by multiple smaller doses.

7. Toca 511/5-FC therapy results in significant increase in survival in peritoneal carcinomatosis models

(a) 100 % pre-transduced SKOV3/IP/Toca511 cells

(b) Reduction in tumor burden demonstrated by in vivo imaging

Toca511/5-FC therapy resulted in significantly increased survival in mouse models of peritoneal carcinomatosis. (a) When tumors were induced with cells 100% pre-transduced with Toca 511 at tumor cell doses of 4e5 and 4e6, followed by 5-FC or PBS cycles (5 days of drug, 9 days no drug) of 500 mg/kg/day. 5-FC cycles were continued for 3 additional cycles for the treatment groups once the PBS control groups had met endpoints (4e6 – 7 cycles, 4e5 – 9 cycles). Significantly increased survival was observed for each tumor dose when compared to the PBS control group for that same dose (4e6: p=0.0027, 4e5: p=0.002). At the lower tumor dose 60% survival was maintained when 5-FC was withdrawn. (b) A reduction in tumor burden in animals administered the lower cell dose followed by 5-FC treatment was clearly shown by IVIS imaging. (c) Importantly significantly increased survival was also observed in animals with established peritoneal carcinomatosis models (4e6 SKOV3IP cells) administered Toca 511 (2x10⁶ TU/mouse) and subsequent cycling of 5-FC.

Conclusions

- Efficient RRV replication in ovarian cancer cells in vitro.
- Toca 511/5-FC mediated cell killing confirmed in vitro.
- RRV replication and spread confirmed in an in vivo ovarian cancer peritoneal carcinomatosis model.
- RRV Dose escalation significantly improved gene delivery efficacy.
- Significant survival benefit observed in peritoneal carcinomatosis models treated with Toca 511/5-FC.

These data indicate that RRV-CD (Toca 511) / 5-FC prodrug activator gene therapy has potential for application to ovarian cancer.

Background

Retroviral replicating vectors (RRV) show distinct advantages as a vector system for gene therapy of cancer:

- Suppression of adaptive immunity and defective innate immunity makes the tumor environment a permissive niche
- RRV permanently integrates into the cancer cell genome: new virions bud off from the cell surface without cytolysis
- RRV can mediate widespread transduction of transgenes to tumors and achieve stable long-term expression in a variety of different cancer models
- RRV carrying prodrug activator genes mediate synchronized cell killing upon prodrgad administration
- Residual infected cancer cells act as a reservoir for viral persistence and re-infection upon tumor recurrence

6. Optimization of vector delivery – Dose escalation

7. Toca 511/5-FC therapy results in significant increase in survival in peritoneal carcinomatosis models

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