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

Sara A. Collins[1], Priyanka Kamath[1,2], Suzanne Matsuura[1], Douglas J Jolly[3], Brian Slomovitz[1,2], Noriyuki Kasahara[1,4]

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

Introduction

Ovarian cancer causes more deaths in the U.S. than any other malignancy of the female reproductive system, and new treatment approaches are needed. Our studies to date have demonstrated dramatic survival benefit when tumor-selective retroviral replicating vectors (RRV) are employed for prodrug activator gene therapy in a variety of preclinical cancer models. In the United States, RRV-mediated gene therapy using yeast cytosine deaminase (CD) is under investigation in multi-center ascending dose trials in patients with recurrent high grade glioma (http://www.clinicaltrials.gov: NCT01156584, NCT01985256, NCT01470794), and based on 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 applying 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.

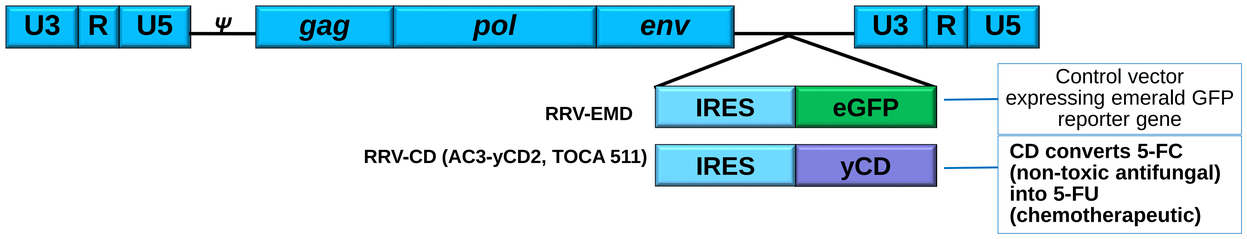
Background

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

- **No nuclear localization signals in RRV capsid; hence RRV infection is intrinsically restricted to dividing cells**
- **Suppression of adaptive immunity and defective innate immunity makes the tumor environment a permissive niche for preferential replication of RRV**
- **RRV permanently integrate into the cancer cell genome: new virions bud off from the cell surface without cytolysis**
- **RRV can mediate widespread delivery of transgenes to tumors and achieve stable long-term expression in a variety of different cancer models *in vivo***
- **RRV carrying prodrug activator genes mediate synchronized cell killing upon prodrug administration**
- **Residual infected cancer cells act as a reservoir for viral persistence and re-infection upon tumor recurrence**

Methods and Results

1. RRV Vectors used in this study



This vector is comprised of a full length replication-competent retrovirus sequence, into which an internal ribosome entry site (IRES)–cytosine deaminase gene cassette has been inserted between the *env* gene and the 3' untranslated region. Ψ, packaging signal; U3/R/U5, domains of viral long terminal repeat; *gag/pol/env*, coding sequences of amphotropic retrovirus.

2. *Cell Lines used in this study*

Established human ovarian cancer cell lines:

- SKOV3-IP – human ovarian carcinoma
- Ovcar5 – human ovarian epithelial carcinoma
- ES2 – human ovarian clear cell carcinoma

Patient Derived Primary Human isolates:

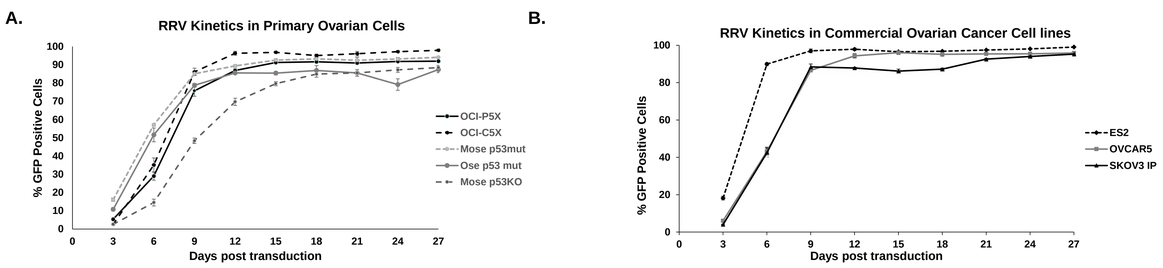
- *C5x – clear cell carcinoma[1]*
- *P5X – papillary serous[1]*

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
- OSEp53mut – Oviduct 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

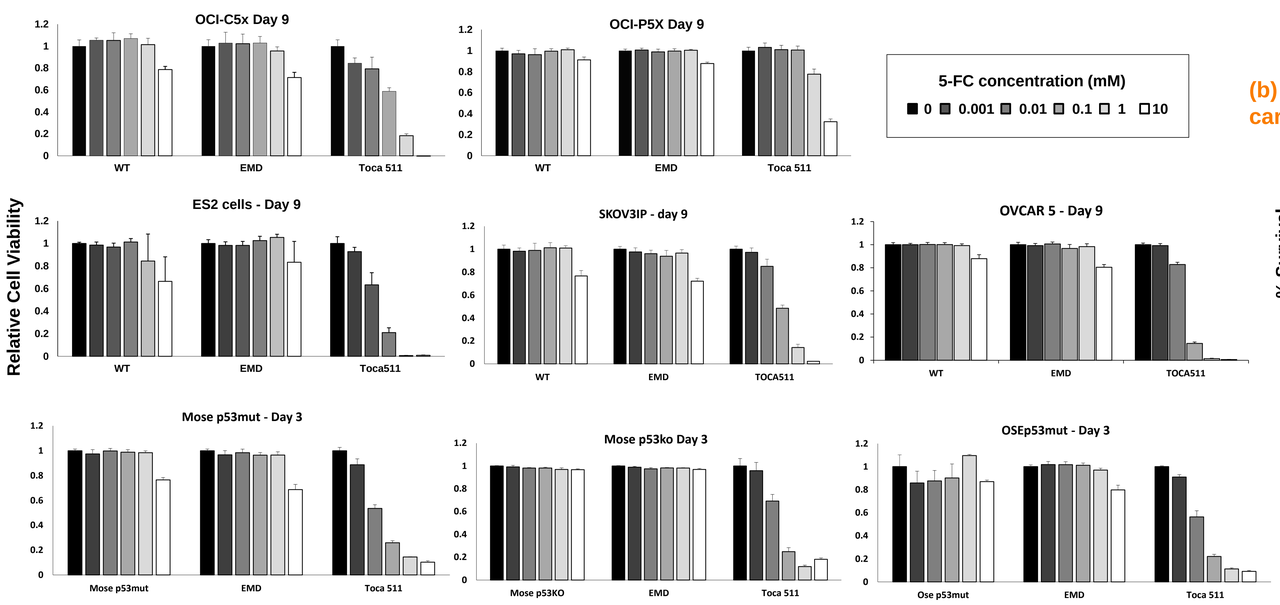
[1]Ince TA., Sousa AD., Jones MA., et al; Characterization of twenty-five ovarian tumor cell lines that phenocopy primary tumours. Nat. Commun. 2015 Jun 17;6:7419

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



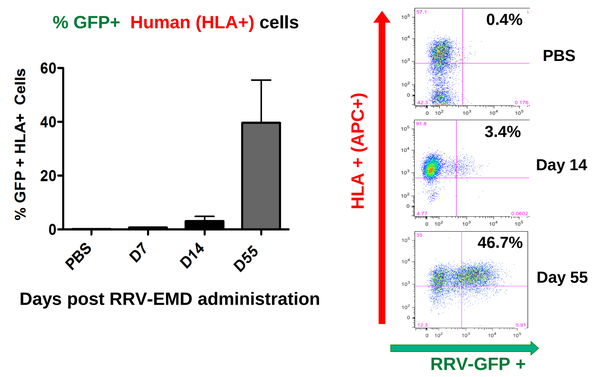
Various ovarian cell lines were transduced with RRV-EMD at MOI = 0.01 and analyzed for GFP expression by flow cytometry every 3rd day after virus infection. RRV spread in (A) Patient derived primary ovarian cancer isolates, (A) Transgenic model-derived primary murine ovarian cancer cells and (B) established 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



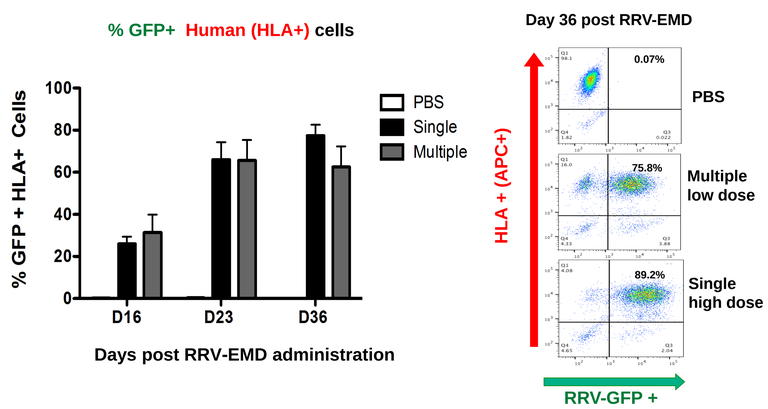
Representative graphs for *in vitro* cytotoxicity assays.
5-FC-mediated killing of transduced ovarian cancer cells. Cells fully transduced with the RRV-EMD or RRV-CD (Toca 511) vectors were seeded at a density of 1 x 10^3 cells/well. 24 hr post-seeding, cells were exposed to 5-FC at 0 mM, 0.001 mM, 0.01 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.

5. Confirmation of RRV transduction and spread in an SKOV3-IP carcinomatosis model



Confirmation of RRV-EMD transduction and spread following IP delivery in an SKOV3-IP peritoneal carcinomatosis model.
7,14 and 55 days post vector (2 x 10^5 TU/mouse in 1 ml) administration, mice were euthanized and tumor cells were isolated from the omentum/mesentery. Briefly tumor cells were dissociated from mouse tissue using collagenase/dispase. Isolated cells were expanded in AZT containing medium to prevent virus spread *in vitro* prior to flow cytometry analysis. For flow analysis, the presence of human cells was confirmed using an APC conjugated human HLA antibody and the number of GFP positive human cells was determined. The % GFP+ human cells (HLA+) isolated from the omentum for n=5 animals is presented in graph. **Isolation of human cells was confirmed as indicated by the shift along the red access when cells were stained with the HLA antibody. GFP+ cells were detected on day 7 post vector administration with an increase in the number of GFP+ cells observed on days 14 and 55.**

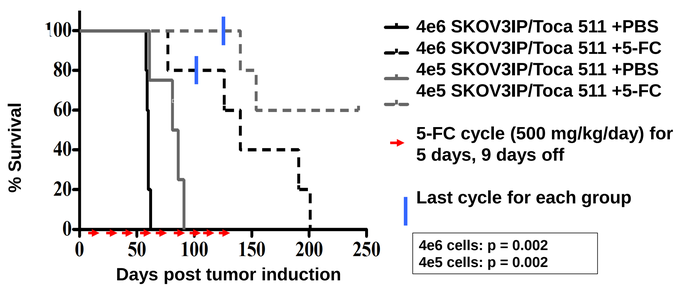
6. Optimization of vector delivery – Dose escalation



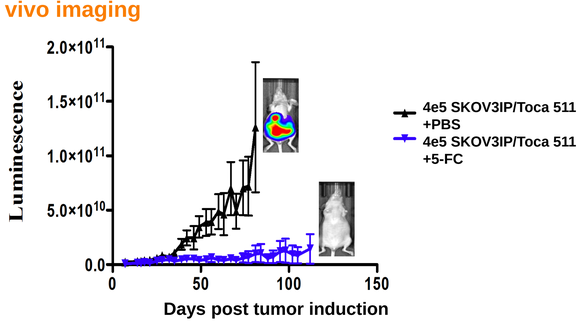
10-fold increase in RRV dose improves efficiency of gene delivery in vivo
Vectors administered IP using two regimens;
Multiple low dose (4 x 10^5 TU/mouse/day in 0.5 ml x 4 days) or Single high dose (2 x 10^6 TU/mouse in 1 ml). On days 16, 23 and 36 post vector administration mice were euthanized and 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 access when cells were stained with the HLA antibody. GFP+ cells were detected on day 16 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 is 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

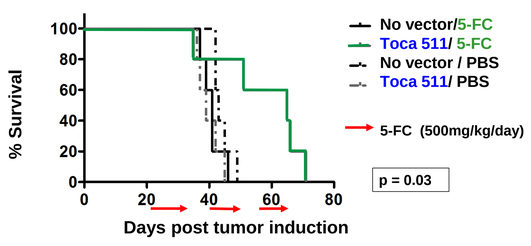
(a) 100 % pre-transduced SKOV3IP/Toca511 cells



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



(b) IP Toca 511 delivery in an established peritoneal carcinomatosis model



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=0.002, 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 IP Toca 511 (2e6 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 efficiency**
- **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.