The University of New Mexico Cancer Center
INST 1115: A PILOT TRIAL TO STUDY THE AVAILIBILITY AND EFFECT OF POST-OP IV KETOROLAC ON OVARIAN, FALLOPIAN TUBE OR PRIMARY PERITONEAL CANCER CELLS RETRIEVED FROM THE PERITONEAL CAVITY

PRINCIPAL INVESTIGATOR:
Carolyn Y. Muller, MD
University of New Mexico Cancer Center
1201 Camino de Salud, NE
Albuquerque, NM 87131
505-925-0460
CMuller@salud.unm.edu

CO-INVESTIGATORS:

Teresa Rutledge, MD
University of New Mexico Cancer Center
1201 Camino de Salud, NE
Albuquerque, NM 87131-0001
505-925-0460
CMuller@salud.unm.edu

Sarah Adams, MD
University of New Mexico Cancer Center
1201 Camino de Salud, NE
Albuquerque, NM 87131-0001
505-925-0395
SAdams@salud.unm.edu

Laurie Hudson, PhD
Regents Professor
University of New Mexico, MSC09 5390,
Albuquerque, NM 87131
505-272-6264
hsc-preaward@salud.unm.edu
505-272-6267

Angela Wandinger Ness, PhD
Professor: Pathology
University of New Mexico, MSC08 4640,
Albuquerque, NM 87131
505-272-1459
AWandinger@salud.unm.edu

Edward Bedrick, Ph.D
Professor: IM Div of Epidemiology
University of New Mexico, MSC10 5550,
Albuquerque, NM 87131
505-272-2520
EBedrick@salud.unm.edu

STUDY LOCATION(S):

University of New Mexico Cancer Center
1201 Camino de Salud, NM
Albuquerque, NM 87131
505-272-4946

University of New Mexico Hospital
2211 Lomas Blvd, NE
Albuquerque, NM 87106
505-272-2111

University of New Mexico College of Pharmacy
2502 Marble, NE Room 179 NRPH Building
Albuquerque, NM 87131
505-272-2482

University of New Mexico Microscopy Fluorescence Facility
University of Mexico School of Medicine
2325 Camino de Salud, NE CRF225A
Albuquerque, NM 87131
505-272-1459

Table of Contents

1.0 BACKGROUND

1.1 The Ovarian Cancer Problem

Ovarian cancer is the sixth most common cancer and the seventh most common cause of cancer deaths in women across the globe.(1) In the US alone, it is estimated that 21,880 new cases of ovarian cancer were diagnosed and 13,850 women died of the disease in 2010. (2) The majority of women, nearly 70%, will present with advanced stage disease that heralds a poor prognosis. (3) Despite aggressive treatment that still favors initial debulking surgery followed by a platinum and taxane based chemotherapy regimen, most patients relapse after achieving a complete clinical response**. (**3, 4)

The management of advanced stage ovarian cancer continues to evolve. A recent randomized trial from the European clinical trials group (EORTC) concludes that neoadjuvant chemotherapy followed by surgical debulking is as effective as primary cytoreductive surgery followed by chemotherapy with less overall toxicity. (5) However, based on some limitations of the trial, most US gynecologic oncologists still favor optimal front line cytoreductive surgery followed by adjuvant chemotherapy. Data from several randomized trials also support the role of intraperitoneal chemotherapy following tumor debulking, demonstrating a significant PFS and overall survival (OS) improvement in patients with optimal surgical cytoreduction (all residual tumor $\leq$ 1 cm). (6) This recent GOG trial (GOG172) led to an NCI alert discussing intraperitoneal chemotherapy as the new standard of care and the GOG has adopted IP therapy as the control arm of the subsequent front-line trial design (GOG 252).(7,8)

There is a move towards personalized medicine in cancer care, and significant effort is underway to evaluate new targeted therapeutics for the treatment of ovarian cancer.(9) Novel therapeutics studied by the Gynecologic Oncology Group (GOG) alone in recurrent ovarian cancer include VEGFR inhibitors (bevacizumab, VEGF-TRAP, sorafenib, enzastaurin, AMG-706), EGFR inhibitors (gefitinib, imatinib, cetuxumab, pertuzumab, tratuzumab, lapatinib), mTOR inhibitors (temsirolimus, everolimus), and DNA methylation inhibitors (vorinostat, dasatinib)[*https://gogmember.gog.org/*]. To date, the only agent of any particular interest is bevacizumab, as recent studies suggest a progression free survival advantage (PFS) in both front line and recurrent ovarian cancer.(10-17) But the cost and toxicity of this agent may not warrant generalized use.(18) The number of novel biologic agents under development is staggering, demanding changes in clinical trial design to efficiently identify promising targets for ovarian cancer.

One means of identifying potential new drug targets is an in-silico approach, where a signaling pathway is chosen for a drug library screen for predicted interaction at a key kinase or enzymatic site. Drug repurposing, screening a library of FDA approved agents, will identify agents that are clinically available and for which pharmacology and pharmacokinetics are known and preclinical data can be generated rapidly without the subsequent need for GMP new drug production. Our co-investigator Wandinger-Ness funded by R03 and R21 grants in collaboration with the UNM Center for Molecular Discovery (UNMCMD) conducted the first comprehensive high throughput screen for inhibitors and activators of small GTPases using purified proteins and libraries of both FDA approved and novel small molecules (19,20). This clinical trial is a novel design in order to test an FDA approved drug for effect on ovarian cancer cells in the peritoneal cavity.

1.2 The Role of GTPase in Ovarian Cancer

Small GTPases, including members of the Rab, Ras and Rho families, are attractive targets for the development of cancer therapeutics based on their pivotal roles in protein trafficking, proliferation/survival and cytoskeletal organization, respectively (Fig. 1). The Ras-homologous (Rho) family of small GTPases (Rac, Cdc42 and Rho) are key regulators of numerous cellular functions including actin reorganization, cell motility, cell-cell and cell-extracellular matrix (ECM) adhesion, all important parameters of ovarian cancer metastatic dissemination ***)*** In many human cancers (including colon and breast), aberrant Rho-family signaling due to changes in the GTPase itself or in its regulation loops is a critical underpinning of tumor growth and survival, invasion and metastasis, [24-26]. Our own studies are the first to demonstrate dysregulation of Rac1 and Cdc42 GTPase expression and activity in ovarian cancer (Fig. 2). Stage and grade dependent overexpression of Cdc42 protein was detected by immunohistochemical staining of human tumor samples (Fig. 2A). Cdc42 overexpression levels were highly significant for malignant, high-grade tumors ($p<0.001$). Although there was more modest elevation of total Rac1 protein, expression of a constitutively active splice variant Rac1b (first documented in breast tumors, [26]) was detected by qPCR (Fig. 2B) and a GLISA activity assay showed both Cdc42 and Rac1 GTPases highly active in fresh tumor isolates, but activity declined after 48h in culture (Fig. 2C). Although Rac1 and Cdc42 have been recognized as attractive therapeutic targets, specific Rac GTPase inhibitors [27,28] are not in clinical use and there are no known Cdc42 specific inhibitors in clinical use.

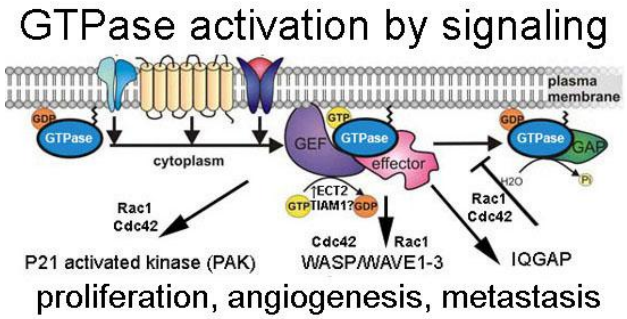


Fig. 1. Rac1 and Cdc42 GTPases integrate signaling pathways that are important in cancer growth and metastasis. Activation of tyrosine kinase receptors, G-protein coupled receptors (GPCRs) and integrins causes Rac and Cdc42 GTPases to bind GTP and membranes. The GTP-bound proteins interact with specific downstream effectors to promote actin reorganization that affect changes in cell motility, adhesion, cell growth, gene expression and apoptosis. GTPase functions in the regulation of proliferation, angiogenesis and metastasis are intimately linked to cancer development and progression. Adapted from [1].

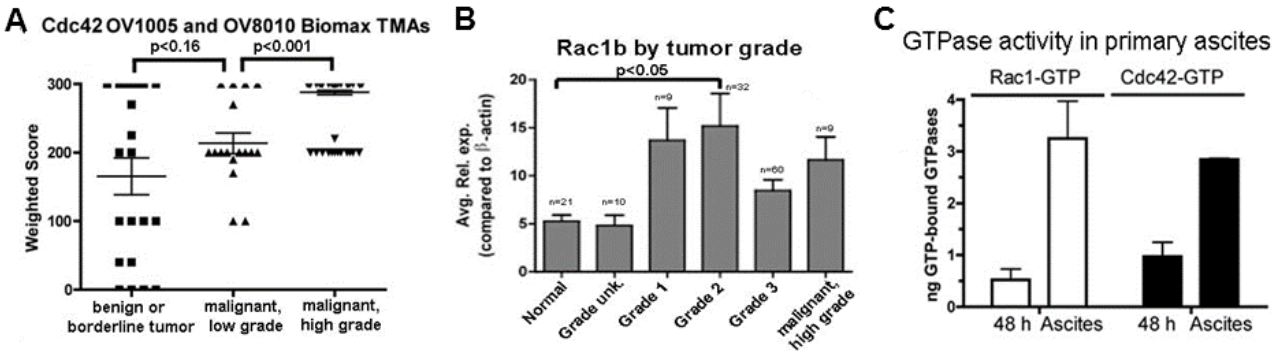


Fig. 2 . Dysregulation of GTPase targets. (A) Ovarian tumor tissue microarrays were purchased from US Biomax and stained for Cdc42 (pAb 10155-1-AP ProteinTech Group, Inc.). Tumor grade and type was confirmed, and immunoreactivity scoring was conducted by pathologist Dr. Lesley Lomo. Statistical analyses show $p<0.001$ for low vs. high grade tumors, UNM Cancer Center Biostatistics core. (B) Primary tumor cDNA samples (TissueScan Disease Tissue qPCR Arrays, OriGene) were screened for Rac1b expression by qPCR. Normal vs. Gr. 2 $p<0.05$. (C) Rac1 and Cdc42 GTPase activities measured by GLISA in cells from fresh ovarian patient ascites or after 48 h in culture.

1.3 Drug Library Screening Predicts R-Entatiomers of Naproxen and Ketorolac as GTPase Inhibitors

Screening of the Prestwick library of FDA-approved molecules identified the R-enantiomer of naproxen as active against Rac1 and Cdc42, which we confirmed in cellular GLISA assays (**Fig. 3A**). R-, and S-ketorolac were identified for structure activity testing based on additional cheminformatics. Despite the common chemotype, S-naproxen and 6-MNA did not significantly inhibit Rac1 or Cdc42 ***indicating that GTPase inhibition is independent of the COX pathway***. At nontoxic doses that are meaningful with respect to human serum levels at therapeutic doses (**Table 1)**, the R-enantiomers of naproxen or ketorolac are more effective for inhibition of migration in OVCA 429 and SKOV3ip (**Fig. 4A-B**), and primary ovarian tumor cells isolated from ascites (not shown). The same structure activity relationship was observed for multicellular aggregate formation (**Fig. 4C**). Inhibition of proliferation was less marked (~50%) with R-naproxen>>S-naproxen. More than 20 other common NSAIDS were tested and did not detect inhibitory activity *in vitro* or in cells against Rac1 or Cdc42, nor any impact on ovarian cell migration or proliferation. *Thus, the structure activity relationships and selectivity suggest that the inhibitory actions of R-naproxen and R-ketorolac are due to an effect on novel Rac1 and Cdc42 GTPase targets as first revealed by the molecular library screen and predicted by cheminformatics.*

Virtual docking predicts that R-naproxen, but not S-naproxen can bind the GDP-bound pocket of Rac1 (**Fig. 3B**). In this model R-naproxen is stabilized through favorable H-bonds with Thr17 and Asp57, as

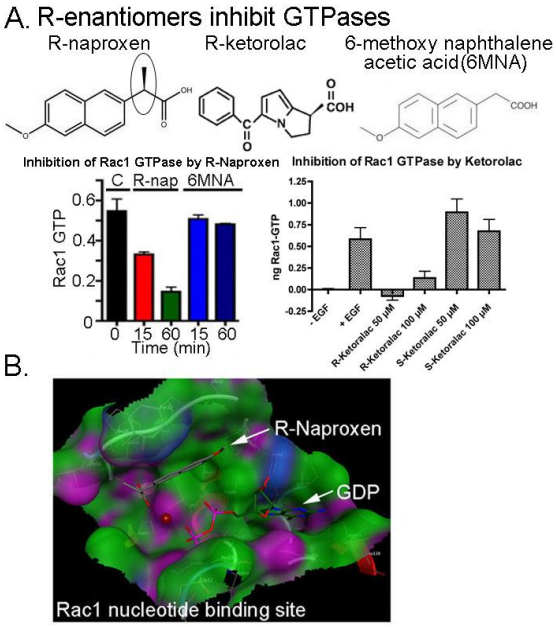


Fig. 3. Enantiomer selective inhibition of GTPases. (A) GLISA measures cellular effects of small molecules on Rac1. Cells were untreated or incubated with the indicated compound (R-Nap=R-naproxen,R-ketorolac, S-ketorolac (enantiomers differ based on orientation of circled methyl group on naproxen or carboxyl group on ketorolac); or the non-stereoselective 6MNA = 6-methoxy-2-naphthalene) for the indicated times then treated +/- 10 ng/ml EGF for 2 min. Equal cell lysate protein was assayed for activated Rac1 using a commercial, plate based Pak-binding assay according to manufacturer's instructions (GLISA, Cytoskeleton, Inc.). Similar results were obtained for Cdc42 and S-naproxen like S-ketorolac was not inhibitory (not shown) **(B) Docking predicts R-enantiomer-selective binding of naproxen to GDP-bound Rac1.** R-naproxen predicted to bind GDP-bound Rac1, but not GTP-bound conformation. S-naproxen is sterically blocked from binding (not shown). Rac1 crystal structure used to dock molecules via FRED (OpenEye).

Table 1. Serum concentrations and effective doses of screened targets					
	S-Nap	**R-Nap**	**(S,R) Ket**	**S-Ket**	**R-Ket**
Target	COX 1/2	Rac1/cdc42	COX 1>2	COX1>2	Rac1/Cdc42
Serum Cmax	413+/-74μM	NA	6.4+/-1.8 μM	NA	NA
Serum Cave	130-391 μM	NA	8.3+/-2.3 μM	NA	NA
IC50COX1	35.5-48.3 μM	>100xS-Nap	0.6 μM	0.1 μM	ND
IC50COX2	64.6-79.5 μM	>100xS-Nap	2.7 μM	2.5 μM	ND
IC50Rac1	>100 μM	TBD	TBD	TBD	TBD
IC50 (est) migration	≥300 μM	100 μM	34 μM	34 μM	7 μM

Legend Table 1. Serum concentrations (maximum (C_{max}) and steady state (C_{ave})) were based on typical oral dosing (S-Naproxen 500 mg; R,S-ketorolac 30 mg) and derived from FDA product literature and primary literature (S-Naproxen, [29,30]; ketorolac [31-35]. Note that an IV dose of 30 mg ketorolac achieves a C_{max} of 13.7 μM [35,36]. IC_{50} values for COX1/2 in human cells were obtained from the literature (R and S-naproxen, [37; 38-40]; R and S-Ketorolac, [31-33; 35; 36; 41]. Migration IC_{50} values were estimated from limited dose response data (Fig. 4) or calculated by GraphPad Prism5 (ketorolac). NA-not applicable, no human dosing; ND=not detected, no COX inhibition; TBD=to be determined.

well as through interaction with magnesium via the naproxen carboxyl group. Interestingly, Thr17 is the mutated residue in dominant negative forms of Rac1. The model predicts stabilization of the inactive GDP-bound GTPase and provides a

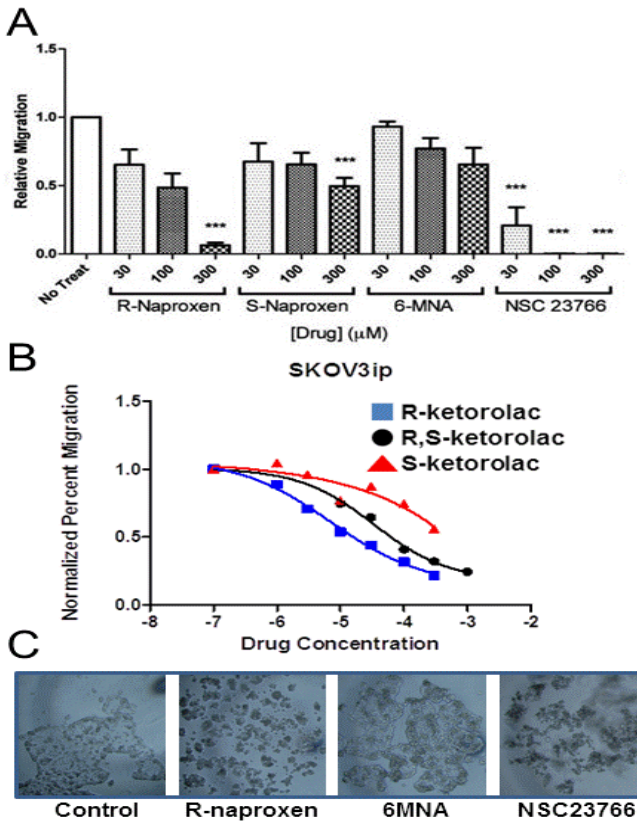


Fig. 4. Enantiomer selective inhibition of ovarian tumor cell migration & aggregation. A) R-naproxen inhibits ovarian tumor cell migration. OVCA 429 cell migration was measured under the indicated conditions using modified Boyden chambers. *P<0.05 Similar results were obtained for SKOV3ip and OVCA 433 cells. **(B) R-ketorolac inhibits ovarian tumor cell migration.** SKOV3ip cells were incubated for 24h under the indicated conditions and migration was measured as in (A). Comparable results were obtained for OVCA 429 cells. **C) Inhibition of aggregation.** OVCA 429 cells were trypsinized and resuspended at 3x106 cells/ml in medium containing drugs as indicated (100 μM). 25 μl drops were suspended and imaged after 24h. Comparable responses were observed in OVCA 433 and SKOV3ip cells.

testable hypothesis for a novel mechanism of action which is under active

investigation. As predicted by the enantiomer-selective inhibition of Rac1 and Cdc42 activity, we find that R-naproxen, R-ketorolac and the established Rac1 inhibitor NSC23766 inhibit ovarian tumor cell proliferation, migration and MCA formation (**Fig. 4A-C** and data not shown) while S-naproxen and 6-MNA display little effect. This data clearly shows the enatiomeric specificity of the target GTPases in ovarian cancer.

1.4 Inhibition of Rac and Cdc2 in Ovarian Cancer – Preclinical Data

Our group has shown that the R-enantiomers of naproxyn and ketorolac inhibit Rac1 and Cdc42 activity which inhibits cell proliferation, migration and multi-cell aggregate formation. This effect is completely independent of any COX inhibition which is a function of the S-enantiomers (Figures 3.4). R-naproxen was used as the prototype drug for the preclinical data, while repeat assays for R-ketorolac are underway.

1.4.1 In vitro GLISA Assays

Our collaborator, Wandinger-Ness, has extensively used GLISA for measuring EGF-stimulated Rac and Cdc42 activation in Swiss 3T3 cells in the presence and absence of test compounds. Preliminary data demonstrate R-naproxen inhibits Rac1 and Cdc42 GTPase activities in OVCA cell lines (429 and 433). The GLISAs are highly specific, quantitative, can be performed with small sample sizes (<50 µg of cell lysate per assay, compared to 1-10 mg required for conventional PAK binding assay) and have excellent reproducibility. Preliminary data demonstrate that R-naproxen, but not 6MNA, inhibits Rac and Tiam membrane association consistent with the inhibition of Rac1 activity measured by GLISA (**Fig. 5A-C**). In addition, the formation of Cdc42-dependent, actin-based invadopodia that are crucial for matrix degradation and invasion [42; 43] is also significantly reduced by R-naproxen, but not by 6 MNA (**Fig. 5D-E**). Repeat studies using R-ketorolac are underway

1.4.2 In vivo Studies

Using R-naproxen as the prototype drug, R-naproxen decreased the number of implanted tumors in a xenograft animal model using athymic nude mice injected with human ovarian tumor cells adapted to intraperitoneal growth (SKOVip; obtained through a formal MTA from MD Anderson). Treatments were incorporated into transgenic dough (BioServe, Frenchtown, NJ) and animals were dosed twice per day to achieve established therapeutic serum levels [39]. Animals were given placebo dough or dough containing R-naproxen, S-naproxen or 6MNA. After sacrifice, animals fed R-naproxen exhibited a 4-fold reduction in tumor number and a 36% decrease in total tumor burden (**Fig. 6**), while the 6MNA and S-naproxen treated animals were similar to the placebo controls. *These data demonstrate that the enantiomer specific inhibitory properties of R-naproxen are preserved in vivo and that this NSAID may reduce tumor growth and spread, a point that will be further tested and extended to ketorolac in this proposal.*

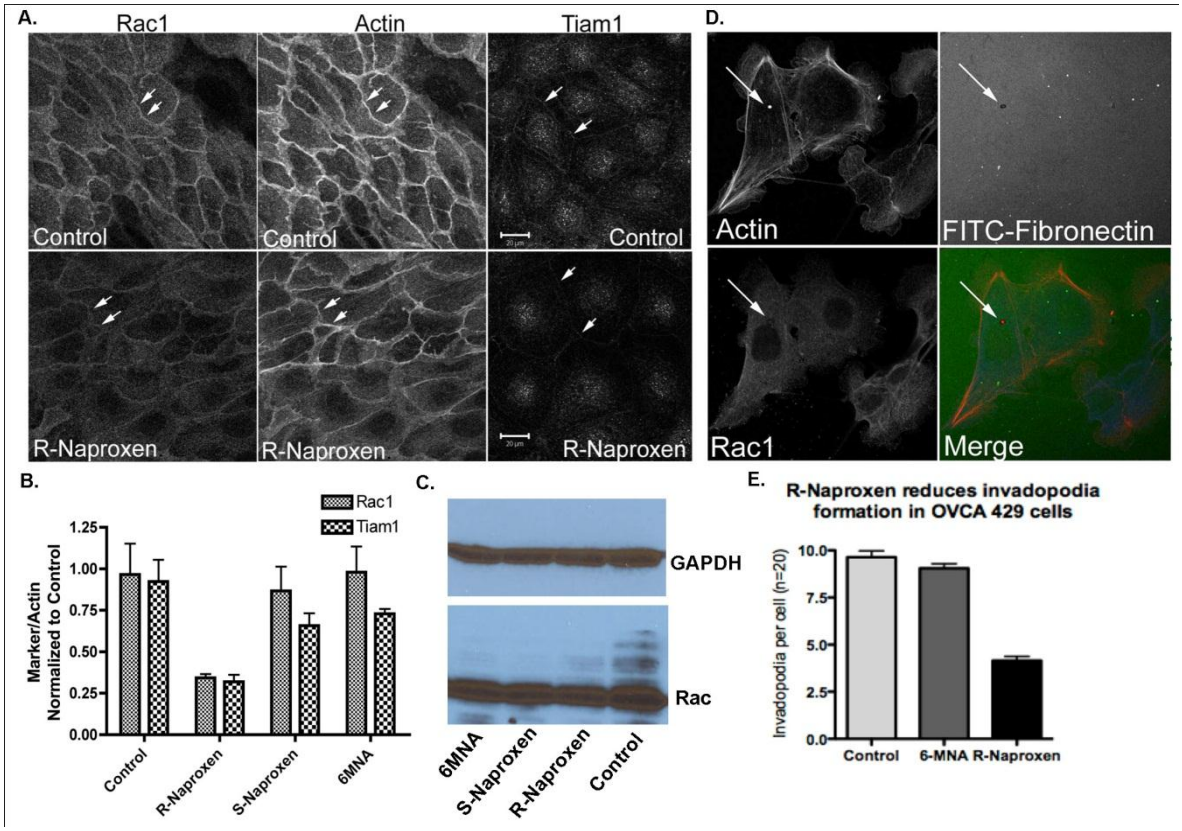


Fig. 5. Assays of GTPase and pathway inactivation by drug treatment. (A) OVCA 433 cells were treated with 100 μM R-naproxen for 1 h, fixed and stained for Rac1, actin and Tiam1. (B) Quantification of staining shows a notable loss of Rac1 and Tiam membrane association with R-naproxen, but not S-naproxen or 6MNA treatment. (C) Total Rac protein levels were unchanged by treatment. (D) OVCA 429 cells plated on FITC-fibronectin extend invadopodia into the matrix and create holes due to the activity of associated matrix metalloproteinases. (E) R-naproxen, but not 6MNA decreased invadopodia formation

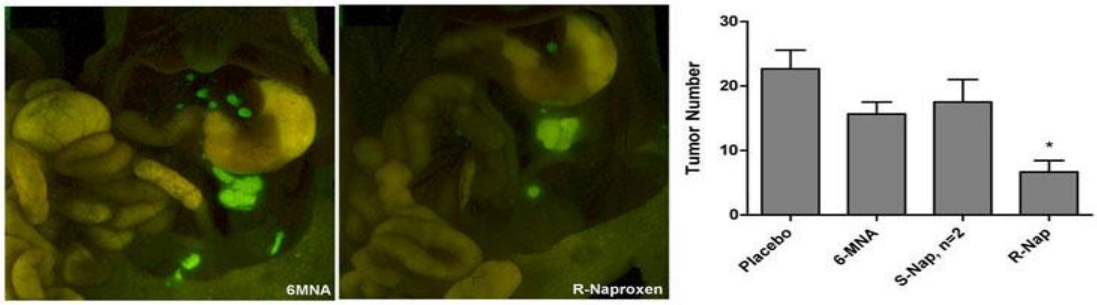


Fig. 6. R-Naproxen reduces tumor number in xenograft model. Athymic nude mice were given an oral dose (10mg/kg) of the indicated compounds in transgenic dough. Individual dosing was confirmed by direct observation. Mice were acclimated with placebo for 3 days and 1 day prior to injections of human ovarian GFP-tagged SKOV3ip cells, mice were left on placebo or provided the indicated treatments. After 2 weeks of tumor growth the mice were sacrificed, necropsy was performed and images were taken of the peritoneal cavity. All tumors were counted. Internal organs were moved to visualize the full cavity with quantification given in the chart.

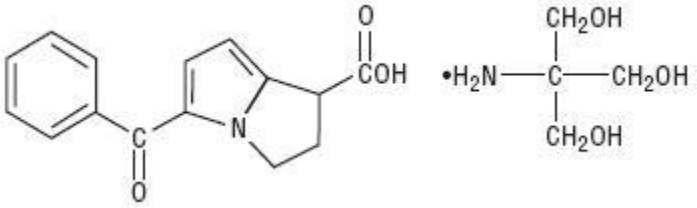
1.5

1.5 KETOROLAC

Ketorolac will be used in this study as preliminary data support the enantiomeric specific inhibition of cell adhesion and migration. Although R-naproxen was the prototype NSAID R-enatiomer, clinically available naproxen is the S-enantiomer. Ketorolac is a racemic mixture of the R and S-enantiomer, the R-enatiomer is stable during metabolism and ketorolac is available for IV administration and is FDA approved for post-operative pain management.

1.5.1. Ketorolac Description

Ketorolac tromethamine is a member of the pyrrolo-pyrrole group of non-steroidal anti-inflammatory drugs (NSAIDs). The chemical name for Ketorolac tromethamine is (±)-5-benzoyl-2,3-dihydro-1H-pyrrolizine-1-carboxylic acid, compound with 2-amino-2-(hydroxymethyl)-1,3-propanediol. The structural formula is:



C19H24N2O6 M.W. 376.41

Ketorolac tromethamine is a racemic mixture of [-]S and [+]R Ketorolac tromethamine. Ketorolac tromethamine may exist in three crystal forms. All forms are equally soluble in water. Ketorolac tromethamine has a pKa of 3.5 and an n-octanol/water partition coefficient of 0.26. Ketorolac is available for oral, intramuscular and intravenous use.

1.5.2. Ketorolac Pharmacology and Pharmacokinetics

Ketorolac tromethamine is a non-steroidal anti-inflammatory drug. The biological activity of Ketorolac tromethamine for pain relief is associated with the S-form. The peak analgesic effect of Ketorolac tromethamine occurs within 2 to 3 hours and is not statistically significantly different over the recommended dosage range of Ketorolac tromethamine. The greatest difference between large and small doses of Ketorolac tromethamine is in the duration of analgesia.

Ketorolac tromethamine is a racemic mixture of [-]S- and [+]R-enantiomeric forms, with the S-form having analgesic activity and the R-form the proposed cancer inhibition effect. The IV dosing of ketorolac is 100% bioavailable. The pharmacokinetic parameters for IV dosing is shown in Table 2. In adults, following IV administration of Ketorolac tromethamine in the recommended dosage ranges, the clearance of the racemate does not change. This implies that the pharmacokinetics of Ketorolac tromethamine in adults, following single or multiple IV doses of Ketorolac tromethamine are linear. At the higher recommended doses, there is a proportional increase in the concentrations of free and bound racemate.

The mean apparent volume (Vß) of Ketorolac tromethamine following complete distribution was approximately 13 liters. This parameter was determined from single-dose data. The Ketorolac tromethamine racemate has been shown to be highly protein bound (99%). Nevertheless, plasma concentrations as high as 10 mcg/mL will only occupy approximately 5% of the albumin binding sites. Thus, the unbound fraction for each enantiomer will be constant over the therapeutic range. A decrease in serum albumin, however, will result in

increased free drug concentrations. The half-life of the Ketorolac tromethamine S-enantiomer was approximately 2.5 hours (SD ± 0.4) compared with 5 hours (SD ± 1.7) for the R-enantiomer. In other studies, the half-life for the racemate has been reported to lie within the range of 5 to 6 hours.

	Tmax (min)	Cmax (mcg/ml) single dose	Cmax (mcg/ml) steady state qid	Cmin (mcg/ml) steady state qid	Cavg (mcg/ml) steady state qid
15 mg	1.1 ± 0.7	2.47 ± 0.51	3.09 ± 0.1.17	0.61 ± 0.21	1.09 ± 0.30
30 mg	2.9 ± 1.8	4.65 ± 0.7	6.85 ± 0.7	1.04 ± 0.7	2.17 ± 0.7
Tmax = time to peak plasma concentration, Cmax = peak plasma concentration, Cmin = trough plasma concentration, Cavg = average plasma concentration					

Table 2. Average Pharmacokinetic Parameters for IV Ketorolac

The intraperitoneal distribution of ketorolac and its racemic mixture is unknown and represents the primary endpoint of this trial. Based on the known pharmacokinetics, it is favorable that the half-life of the R-enantiomer is longer and that both are protein bound. We anticipate that the post-op decrease in serum albumin will favor free drug with a high rate of transport into the peritoneal cavity.

1.5.3 Side Effects from Ketorolac

There are few side effects that are expected after a single dose. Side effects that are likely in 1% -10% chance include: pain at the IV site, rashes, itching, abdominal pain, gas, bleeding, mouth sores, constipation, diarrhea, bloating, heartburn, vomiting, upset stomach, ulcers, high liver function tests, low red cell count, swelling, increased bleeding time, purple spots, drowsiness, ringing in the ears, dizziness, sweating and abnormal kidney function. Side effects that have a greater than 10% chance of happening include: nausea and headaches. It is very unlikely that these side effects will occur from a single dose or even several doses over 24 hours. Many of these side effects will also be present just from the surgery and other medications given after the surgery.

1.5.4 Side Effects from Blood Tests or Intravenous Risks

Frequent blood samples will be collected during this study. The subject may experience pain, bleeding from the puncture site or in tissues surrounding the puncture site, blood clot formation, or local infection and inflammation at the puncture site (very rare). An intravenous catheter (plastic tube) will be in place for routine postoperative care and will be the site used for administration of the ketorolac. It is typical that a second IV is in place. If able, this IV will be used to draw the study samples in an attempt to avoid additional needle sticks. In the event that a second IV is not in place, the investigator will draw blood using a small needle. Bleeding at the site where the catheter is or where needle sticks are made into the skin can cause bleeding under the skin causing a bruise. There is a possibility of infection, with swelling, redness and pain at the site where the ketorolac goes into the vein. It is extremely rare but possible that a serious infection of the blood stream or the heart valves or the formation of a blood clot in the lungs. The subjects will be re-admitted to the hospital to get treatment for these rare but serious problems if they occur after hospital discharge.

1.5.5 Side Effects from *Peritoneal Port*

Frequent fluid samples from the subjects' abdomen will be collected during this study. The subject may experience pain, bleeding from the puncture site at the port or in tissues

surrounding the puncture site, blood clot formation, or local infection and inflammation at the puncture site (very rare). A Huber needle is inserted in the port reservoir at the time of catheter placement in the operating room and left there during the time of the collection.. The subject may feel some irritation in abdomen during the time when the fluid is being drawn into a syringe. There is a very small possibility that they subject will develop peritonitis. This would need to be treated with antibiotics and would likely become evident during the hospitalization.

1.5.6 Potential Benefits

Ketorolac will improved pain control in the post-operative period. It is also possible that use of ketorolac in ovarian cancer patients may have a benefit in cancer outcome as is suggested in one breast cancer study.

2.0 OBJECTIVES

2.1 Primary Objective

The primary objective of this study is to determine the concentration of measurable levels of R and S- Ketorolac (and the percent racemic mixture) in the peritoneal cavity after IV administration in the post-operative patient after cytoreductive surgery.

2.2 Secondary Objectives

The secondary endpoint of this study is to determine the effect of IV ketorolac on cell adhesion and migration of ovarian cancer cells retrieved from the peritoneal cavity after cytoreductive surgery

.

3.0 INCLUSION OF WOMEN AND MINORITIES

The University of New Mexico Cancer Center will not exclude potential subjects from participating in this or any study solely on the basis of ethnic origin or socioeconomic status. Every attempt will be made to enter all eligible patients into this protocol and therefore address the study objectives in a patient population representative of the entire ovarian, fallopian tube, and primary peritoneal cancer population treated by participating institutions. Only women will be recruited to this trial as only women can be diagnosed with ovarian, fallopian tube or primary peritoneal cancer.

4.0 PATIENT ELIGIBILTY AND EXCLUSIONS

4.1 Inclusion Criteria

This protocol will have a 2 stage eligibility as consent will be done in the preoperative planning. Patients must meet all of the following criteria to be eligible for study entry.

Primary Eligibility (pre-op)

- Patients must be suspected of having a diagnosis of ovarian, fallopian tube or primary peritoneal cancer with a planned cytoreductive surgery.
- Borderline ovarian cancer with ascites.
- ECOG/Zubrod/SWOG Performance Status <2 (Karnofsky Performance Status $\geq$ 70%)
- Female, age $\geq$18 years
- Ability to provide informed consent
- Baseline laboratory values (bone marrow, renal, hepatic):
    - Adequate bone marrow function:

        - Absolute neutrophil count >1000/μL
        - Platelet count >100,000/μL
    - Renal function:
        - Serum creatinine ≤ 1.5 x ULN
    - Hepatic function:
        - Bilirubin ≤1.5x normal
        - Serum glutamic-oxaloacetic transaminase (SGOT) (aspartate aminotransferase [AST]) or serum glutamic-pyruvic transaminase (SGPT) (alanine aminotransferase [ALT]) levels ≤2 x ULN
- No known bleeding disorders
- No known sensitivity to NSAIDs
- No active peptic ulcer disease
- No active bleeding

Secondary Eligibility

- Histologic diagnosis of epithelial ovarian, fallopian tube or primary peritoneal cancer on frozen section diagnosis
- Placement of an intraperitoneal port at the time of surgery for anticipated use for adjuvant chemotherapy or management of post-operative ascites
- If epidural catheter was used – the catheter must be removed prior to treatment
- No active bleeding

4.2 Exclusion Criteria

Patients meeting any of the following criteria are ineligible for study entry:

- Non-epithelial ovarian cancer or metastatic cancer to the ovaries
- Borderline ovarian cancer without ascites
- Presumed early stage ovarian cancer
- No clinical indication for a peritoneal port
- Active use of an epidural catheter
- Uncontrolled or unstable medical conditions
- Off study use of ketorolac or other NSAIDs prior to study administration
- Active bleeding or high risk of bleeding
- Active therapeutic anticoagulation
- Known hypersensitivity to NSAIDs
- Chronic or acute renal insufficiency as defined by a preoperative serum creatinine greater than 1.5mg/dL or creatinine clearance of < 60 ml/min
- Any co-morbid condition that, in the view of the attending physician, renders the patient at high risk from treatment complications

5.0 STUDY DESIGN

5.1 Study Design

This is a pilot (feasibility) trial to determine the availability, concentration and racemic mixture of a clinically indicated pain medication, ketorolac, in the peritoneal cavity and the subsequent effect on free floating ovarian cancer cells in patients after cytoreductive surgery for ovarian cancer.

5.2 Study Population

Adult female subjects at least 18 years of age suspected of having a diagnosis of ovarian, fallopian tube or primary peritoneal cancer with a planned cytoreductive surgery will be eligible for this study.

Subjects will undergo a pre-operative and a post-operative eligibility evaluation. Subjects who meet all of the inclusion criteria and do not meet any of the exclusion criteria will be eligible for enrollment into the study.

6.0 SUBJECT INFORMATION AND CONSENT

The Investigator or her representative will explain the nature of the study to the subject, and answer all questions regarding this study. Prior to any study-related pre-operative and post-operative procedures being performed on the subject, the informed consent statement will be reviewed and signed and dated by the subject and the person who administered the informed consent. A copy of the informed consent form will be given to the subject and the original will be placed in the subject's medical record.

Spanish speaking subjects will be consented using a Spanish Short Form. A Certified Spanish Translator from the University of New Mexico will translate the study processes and procedures to the subject and family member.

The subjects will be re-consented if revisions are made to the consent form that would alter the subjects decision to participate in the trial.

7.0 STUDY RECRUITMENT PROCESS

The Principal Investigator, Dr. Carolyn Muller and Co-Investigator, Dr. Teresa Rutledge will identify potential study participants through medical record review from patients within their practices. The Investigators will discuss this study with subjects during the subjects pre-operative counseling visit.

Subjects will not be compensated for participation in the research study.

8.0 STUDY MODALITY

8.1 Ketorolac tromethamine (Torodol® Roche Pharmaceuticals)

Formulation: Ketorolac is supplied in sterile single use 1mL vials of 15 mg/mL(**NDC 55390-480-01)** and 30 mg/mL, 1 mL single-dose vials of 30mg/mL (**NDC 55390-481-01).** Supplies are obtained by the University Hospital (University of New Mexico) and stored in the Pyxsis until ordered by the treating physician.

Preparation: Ketorolac tromethamine for IV use is supplied in 1 mL vials of either 15mg or 30mg individual dose which requires no reconstitution.

Storage: Vials of ketorolac tromethamine are stored at room temperature protected from light in the carton until use.

Dosing: Ketorolac tromethine will be dosed according to FDA approved recommendations. For planned ***single dose (IV)*** based on clinical scenario:

- Patients < 65 years of age: One dose of 30 mg.
- Patients ≥ 65 years of age, renally impaired and/or less than 50 kg (110 lbs) of body weight: One dose of 15 mg.

For planned ***Multiple-Dose Treatment (IV)*** for indicated pain management

- Patients < 65 years of age: The recommended dose is 30 mg ketorolac tromethamine injection every 6 hours. The maximum daily dose for these populations should not exceed 120 mg.

• For patients ≥ 65 years of age, renal impaired patients, and patients less than 50 kg (110 lbs): The recommended dose is 15 mg ketorolac tromethamine injection every 6 hours. The maximum daily dose for these populations should not exceed 60 mg.

Adverse Effects: Complications of treatment with ketorolac tromethamine, such as G.I. ulceration, bleeding and perforation, postoperative bleeding, acute renal failure, anaphylactic and anaphylactoid reactions and liver failure have been described. These NSAID-related complications can be serious in certain patients for whom ketorolac tromethamine is indicated, especially when the drug is used inappropriately.

In patients taking ketorolac tromethamine or other NSAIDs in clinical trials, the most frequently reported adverse experiences and frequency are shown in the table below:

	< 1%	**1%-10%**	**>10%**
cardiovascular	CHF, palpitation, pallor, tachycardia, syncope	hypertension	
dermatologic	Alopecia, urticaria, photosensitivity	Injection site pain, rashes, pruritis	
gastrointestinal	Anorexia, dry mouth, eructation, esophagitis, excessive thirst, gastritis, glossitis, hematemesis, hepatitis, increased appetite, jaundice, melena, rectal bleeding	Abdominal pain, flatulence, bleeding, perforation, stomatitis, constipation, diarrhea, GI fullness, heartburn, vomiting, dyspepsia, ulcers, elevated liver functions	nausea
heme and lymph	Ecchymosis, eosinophilia, epistaxis, leukopenia, thrombocytopenia	Anemia, edema, increased bleeding time, purpura	
metabolic/nutritional	Weight change		
nervous system	abnormal dreams, abnormal thinking, anxiety, asthenia, confusion, depression, euphoria, extrapyramidal symptoms, hallucinations, hyperkinesis, inability to concentrate, insomnia, nervousness,	Drowsiness, tinnitus, dizziness, sweating	headaches

	paresthesia, somnolence, stupor, tremors, vertigo, malaise		
reproductive	infertility		
respiratory	Asthma, cough, dyspnea, pulmonary edema, rhinitis		
urogenital	cystitis, dysuria, hematuria, increased urinary frequency, interstitial nephritis, oliguria/polyuria, proteinuria, renal failure, urinary retention	Abnormal renal function,	
others	Fevers, infections, sepsis, conjunctivitis		

Table 3*. Reported toxicities of racemic ketorolac*

Supplier: Manufactured by Bedford Laboratories™ Bedford, OH 44146 Div-KRL-P05. Vials are supplied directly to University Hospital.

Administration: Ketorolac 15mg or 30 mg doses will be administered via IV Push undiluted over 15 – 30 seconds. Prior to administration, the IV line will be flushed with 5-10 ml's NS. The ketorolac dose will be followed with a 5-10 ml NS push again to ensure the ketorolac completely clears the IV line.

9.0 TREATMENT PLAN

All patients will receive a single dose of IV ketorolac for pain management for the indication of post-operative pain control. The single dose will be considered the study dose for which all consenting eligible patients will receive. The dose of IV ketorolac will be determined per the recommendations in the package insert. Patients less than 65 years of age and in otherwise good health will receive a 30mg IV single dose. Patients 65 years of age or greater, or who have mild renal insufficiency or are of low weight (as per section 3.2) will receive a single 15 mg dose IV ketorolac. In patients who have a clinical pain response and have no contraindications to multi-dose (every 6 hours over 24 hours), additional doses will be given per physician discretion based on clinical indication. Patients receiving 24 hour dosing will be eligible for sample time points after 24 hour dosing.

9.1 Concomitant and Excluded Therapies

Use of other non-steroidal (NSAIDs) medications during the time-points of this trial is not permitted while participating in this study. Narcotic regimens with or without acetaminophen is allowed. If patients require additional NSAIDs for pain control, the patients will be withdrawn from study although data collected prior to withdrawal will be included in the analysis. Use of cytotoxic chemotherapy during this trial is not allowed, however if the clinical situation warrants immediate chemotherapy, the patient will be withdrawn from the trial. Prophylactic anticoagulation is allowed but therapeutic anticoagulation is a direct exclusion criteria.

Use of concurrent investigational agents is not permitted.

9.2 Patient Discontinuation

Patients may discontinue study treatment and sample collection at any time. The primary reason for discontinuation should be recorded. Reasons for discontinuation of a patient by the investigator include, but are not limited to, the following:

- Need for additional use of ketorolac or other NSAIDs over the outlined collection period
- Clinically significant deterioration of the patient's condition during the study
- Patient noncompliance
- Peritoneal port failure
- Development if intra-abdominal complication
- Development of infectious morbidity
- Acute blood loss
- Investigator determination that it is not in the patient's best interest to continue participation

9.3 Study Discontinuation

It is unlikely that the trial itself will require a premature termination. However, the trial will be stopped prematurely (prior to completion of accrual, protocol therapy, and follow-up) for safety reasons.

10.0 STUDY ASSESSMENTS

All samples from the peritoneal cavity will be retrieved by the PI (Dr. Muller) or co-PI 's (Dr. Rutledge and Dr. Adams) gynecologic oncologists who are skilled in the access and management of newly placed intraperitoneal ports. The ports will ideally be accessed at the time of surgery and access maintained until completion of the study endpoint samples.

10.1 Pre-treatment Sample Collection

- Collection of ascites cells at the time of tumor debulking
- Collection of peritoneal fluid and cells by the PI (Dr. Muller) or the co-PI's (Dr. Rutledge and Dr. Adams) and blood prior to ketorolac administration.

10.2 Post-treatment Sample Collection

- Collection of peritoneal fluid after ketorolac administration will be done at select time-points after ketorolac administration (1 hour, 6 hours, and 24 hours) an additional 48 hour sample (after the initial dose) will be done in patients having elective multiple ketorolac dosing.
- Cells and effluent will be separated from fluid at the above select time-points (1 hour, 6 hours, 24 hours) and at 48 hours after the initial ketorolac dose in patients who receive elective multiple ketorolac dosing.

10.3 Pharmacokinetic Evaluation:

Blood and 10-20 ccs of peritoneal fluid will be retrieved at the following time-points. Serum will be isolated and peritoneal fluid will be isolated from cellular material via centrifugation.

Timepoint	Time after ketorolac
1	Predose
2	1 hour
3	6 hours
4	24 hours
5	48 hours*

** elective time-point for patients receiving clinically indicated multiple dosing of ketorolac*

10.3.1 Ketorolac Concentration in Peritoneal Fluid - HPLC

Peritoneal concentrations of the R(-) and S(+) enantiomers of ketorolac will be determined by using published procedures for measuring plasma concentrations (44). High pressure liquid chromatography will be performed using UV detection at 313 nm. Standards will be prepared at 7 ketorolac plasma concentrations using 100 mcL steroid-free plasma to attain final concentrations of 0.025, 0.05, 0.10, 0.25, 0.5, 1.0, and 2.0 mcg/mL. Peritoneal samples (100 mcL) and standards will be added to silanized tubes containing the internal standard, loxapine HCL. Before extraction with 4 mL of methyl tert-butyl ether, proteins will be precipitated with 30 mcL of 10% weight to volume solution of trichloroacetic acid. Samples will be shaken for 10 min, centrifuged for 10 min, and the organic layer transferred to silanized culture tubes and evaporated under nitrogen. Mobile phase (50 mcL) will be added, the samples then will be transferred to autosampler vials, and 30 mcL will be injected onto the high pressure liquid chromatography column. Baseline separation of the isomers will be achieved using a chiral column (Astec Chirobiotic R (250 _ 4.6 mm2) obtained from Advanced Separation Technologies, Inc. Whippany, NJ) using a binary gradient mobile phase of 0.07 g ammonium formate and 24 mcL glacial acetic acid per liter methanol and increasing to 100% methanol for 17.5 min at a flow rate of 1.0 mL/min. Samples will be performed in duplicate.

10.3.2 GTPase Inhibitory Activity of Peritoneal Fluid

Rac1 activity will be measured in cells using effector binding assays (GLISA) that are commercially available (Cytoskeleton, Inc) as an independent measure that there is sufficient bioactive R-ketorolac in the peritoneal fluid to inhibit GTPase activity in cultured cells (as illustrated in Fig 3A, ketorolac panel). Because Rac1 inhibition is most robust we will focus on this GTPase. 3T3 cells will be cultured without serum in 6-well dishes in duplicate and subsequently incubated with 500mcL/well peritoneal fluid from each time-point for 1 hour prior to stimulation with 10ng/ml EGF for 2 minutes 3T3 cells will be harvested, lysed and assayed for activated Rac1 GTPase per manufacturer's instructions. Results will be reported as ng Rac1-GTP normalized to +EGF drug samples. Controls will be included to monitor EGF responsiveness of Rac1 activation and ketorolac inhibition of Rac1 activation. Negative controls: peritoneal fluid prior to ketorolac treatment and cells without EGF stimulation. Positive controls: 1 ng purified Rac1-GTP; +EGF treated cells with serum in lieu of peritoneal fluid; cells treated with 100uM R-ketorolac or S-ketorolac for one hour as shown in Fig. 3A.

10.3.3 Efficacy of Peritoneal Fluid containing Ketorolac in Inhibiting Cell Adhesion and Spreading in Vitro.

The peritoneal fluid will be tested on immortalized cells in vitro as an alternate measure that there is sufficient bioactive R-ketorolac in the fluid to inhibit the adhesion/migration of immortalized mouse Swiss 3T3 or human ovarian cancer cells (OvCa429, SKOV or OvCa433) in culture. The assay will utilize CYTOO micropatterned chips that are coated with fibronectin in defined patterns (144 micropatterns/sector with 12 sectors/chip) (**Fig. 7**). Immortalized cells will be mixed with 500 mcL/5x10^3 immortalized cells that have been

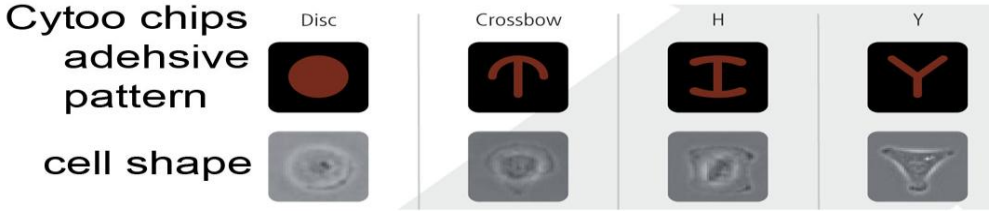


Fig. 7. Cytoo micropatterened chips for analysis of cell adhesion and spreading. Chips have arrays of adhesive patterns as illustrated in red that enable cells to adhere and spread into characteristic shapes. The chips have 144 micropatterns/sector, there are four different shapes arrayed in three sizes (small, medium and large). Thus, a single chip allows the analysis of >1700 cells. We have tested cell shape changes in response to EGF stimulation +/- a Cdc42 inhibitor. Inhibition of Cdc42 caused cells to look like unstimulated control cells, and fail to adopt a migratory phenotype + EGF that was readily quantified using the chips (not shown).

trypsinized and washed with PBS^{+}. The mixture will be allowed to adhere to a CYTOO chip for 15-60 min. Cells will be fixed directly, imaged and evaluated for cell number and cell shape on each of four micropatterns. It is expected that the presence of R-ketorolac will reduce speed of cell adhesion and or cell spreading on the substrate. Positive controls: peritoneal fluid prior to ketorolac treatment; cells in serum in lieu of peritoneal fluid; Negative controls: cells plated in the presence of 100 μM R-ketorolac or S-ketorolac for one hour prior.

10.3.4. Spreading, Adhesion, and Migration Assays of Patient Derived Peritoneal Cells

The impact of ketorolac treatment on residual ovarian tumor cell behavior will be assessed by measuring time for cell adhesion and spreading, as well as migration distance as follows. All of the assays require only small numbers of cells. CYTOO chips (see 5.6.5 and Fig. 7) will serve as an alternate measure for assessing cell adhesion and spreading. Controls: patient cell adhesion prior to treatment; cell adhesion when incubated with 100 μM R-ketorolac.

10.3.4.1. Spreading: Cells (100-5,000/sample) will be rinsed in Dulbecco's PBS (DPBS) and plated on coverslips without or with extracellular matrix coating and images will be obtained at regular intervals. Spreading is defined as flattened cells without the refractile, fibroblastic morphology determined and expressed as a percentage of the total cells ± SD.

10.3.4.2. Adhesion: Cells (100-5,000/well) will be plated onto 96 well plates without or with extracellular matrix coating and incubated for 30 min. Cells will be fixed and non-adherent cells will be removed by gentle washing. Colorimetric quantification of adherent cells will be performed using standard protocols.

10.3.4.3 Migration: Cells (100-5,000/sample) will be placed on collagen-coated coverslips in culture medium and migration will be measured by time lapse photography on a heated microscope stage (UNM Microscopy Facility). Distance traversed for individual cells will be quantified by image analysis software.

10.3.5. PCR analysis of Patient Derived Peritoneal Cells

Genes downstream of Rac1 and Cdc42 activation, which have been validated in cell culture and through in vivo studies (e.g. PAK, IQGAP, DOCK-180, CRK, WAVE3) will be tested by quantitative real time PCR using human genome predesigned and validated primer sets and assay kits from Origene (Rockville, MD). Each assay will be performed in duplicate and ketorolac response will be established by comparison of pretreatment versus treatment samples. A >2-fold change between 0 treatment and subsequent treatment time-points

would be considered significant. This assay will be performed only when sufficient cell numbers are present in the peritoneal fluid (>10,000 cells) to allow purification of RNA.

11.0 STATISTICAL METHODS

11.1 Analysis of Demographic and Baseline Characteristics

The analysis of demographic characteristics (age, gender, tobacco abuse history and ethnicity) and baseline characteristics, including weight change, performance status, and histologic subtype, will be primarily descriptive.

11.2 Endpoint Analyses

11.2.1 Primary Endpoint and Sample Size

Ketorolac availability, concentration and racemic mixture of Toradol® ([R,S]-ketorolac) in the peritoneal cavity. Intraperitoneal concentration and racemic mixture of ketorolac is currently unknown although serum pharmacokinetic data suggests a slower clearance of the R-racemate. The **primary objective** of this study is to determine the concentration of measurable levels of R and S- Ketorolac (and the percent racemic mixture) in the peritoneal cavity after IV administration in the post-operative patient after cytoreductive surgery. Since this is a pilot trial for feasibility of obtaining the desired measures, a true sample size calculation is difficult to assess. We anticipate that we will need to recruit 60 patients pre-operatively who meet the first phase of eligibility. We anticipate that 60% will have a diagnosis other than advanced stage invasive epithelial ovarian cancer, fail optimal debulking, have a clinical decision to not proceed with a port placement or not enough ascites or negative cytology of the ascites that will warrant no further participation in the study. This data is based on our initial experience with the first 12 patients enrolled on the trial. We then anticipate that 25 patients will have intraoperative port placement and be eligible for secondary eligibility. We anticipate that and additional 15-18 patients will either fail secondary eligibility, decline sample collection, decline ketorolac or have indications to withhold ketorolac, decline blood sampling, have inadequate re-accumulated fluid for sampling, or failed extraction of ascites samples from the port. The sample target remains 7-10 complete sets of blood and ascites samples for primary and secondary endpoint analyses.

Data will be analyzed as a repeated measure ANOVA followed by Tukey's post hoc analysis in order to evaluate whether there are significant differences between groups (pre-and post-ketorolac treatment). We plan to group both dose groups together as we anticipate similar drug measures based on known prior serum pharmacokinetic studies. If we find a large discrepancy in peritoneal ketorolac concentrations between the 15mg and 30mg treated group, we may amend the protocol to increase sample size to evaluate the groups independently. The analysis of demographic characteristics (age and ethnicity) and clinical characteristics, including histologic tumor type, and stage of disease will be descriptive. For the peritoneal distribution study, seven patients should be sufficient for statistically meaningful results based on a study of similar design to measure concentration of doxorubicin in ovarian ascites following IV administration (45).

11.2.2 Secondary Endpoints

We will determine the effect of IV ketorolac on cell adhesion and migration on ovarian cancer cells retrieved from the peritoneal cavity after cytoreductive surgery using several different biologic assays. Isolated cells will be tested for evidence of target inhibition and functional changes in adhesion and migration. Target activity assays: Rac1 and Cdc42 activity will be measured in cells using effector binding assays (GLISA) that are

commercially available (Cytoskeleton, Inc) and are used to determine activity of primary cells ex vivo as we have demonstrated in (Fig. 1C). In addition, we will conduct qRT-PCR analysis of cells isolated pre and post ketorolac administration to test for expression of genes downstream of Rac1 and Cdc42 activation that were validated in cell culture and in vivo studies. Adhesive properties of cells will be measured by assays for spreading and adhesion. For spreading analysis, cells will be rinsed in Dulbecco's PBS (DPBS) and plated on coverslips without or with extracellular matrix coating and images will be obtained at regular intervals. Spreading is defined as flattened cells without the refractile, fibroblastic morphology determined and expressed as a percentage of the total cells ± SD as we've described previously (46). For adhesion, cells will be plated onto 96 well plates without or with extracellular matrix coating and incubated for 30 min. Cells will be fixed and non-adherent cells will be removed by gentle washing. Colorimetric quantification of adherent cells will be performed using standard protocols. Migration will be measured after plating cells on collagen-coated coverslips in assessing migration by time lapse photography on a heated microscope stage (UNM Microscopy Facility). Distance traversed for individual cells will be quantified by image analysis software**.** Data will be analyzed as a repeated measure ANOVA followed by Tukey's post hoc analysis in order to evaluate whether there are significant differences between groups (pre-and post-ketorolac treatment). Trend analyses of response will also be performed based on peritoneal R-ketorolac concentrations.

11.3 Safety Analysis

Patients will be examined at each time-point for subjective/objective evidence of developing drug toxicity according to NCI-CTC toxicity criteria or complications from the blood draws or port access

12.0 SOURCE DOCUMENTS AND CASE REPORT FORM COMPLETION

12.1 Source Documents

Source documents are defined as original documents, data and records. This may include hospital records, clinical and office charts, laboratory data/information or evaluation checklists, pharmacy dispensing and other records recorded from automated instruments. Data collected during this study must be recorded on the appropriate source documents.

12.2 Case Report Forms

Case report forms (CRF) must be completed for each subject enrolled in this study. The case report forms will contain the subjects unique indentifiers, demographic characteristics (age, gender, and ethnicity) and baseline characteristics, including vital signs, performance status, and histologic subtype, will be primarily descriptive The information on the case report forms will be collected and used for data analysis.

Information obtained from subjects will be used by study staff and it will be shared with the sponsor of the study (representatives of the Department of Defense). The University of New Mexico Health Sciences Center Human Research Review Committee (HRRC) which oversees human subject research, the New Mexico Cancer Care Alliance, the UNM CC Clinical Trials Office, and the Food and Drug Administration and/or other entities that may have access will be permitted to access to records.

Study records and specimens will be assigned a code number by the study team and the subjects will not be identified by name in the study records.

Study records will be retained for 2 years following the date of study completion.

13.0 ASSESSMENT OF SAFETY

The safety of this therapy will be assessed through collection and analyses of adverse events (AEs) and laboratory tests.

13.1 Specification of Safety Variables

Safety assessments will consist of monitoring and recording protocol-defined adverse events (AEs) and serious adverse events (SAEs); measurement of clinically-specified hematology, clinical chemistry, and urinalysis variables; measurement of clinically-specified vital signs; and other clinically-specified tests that are deemed critical to the post-operative patient care plan.

13.1.1 Adverse Events

An AE is any unfavorable and unintended sign, symptom, or disease temporally associated with the use of any medication or other protocol-imposed intervention, regardless of attribution.

This includes the following:

- AEs not previously observed in the patient that emerge during the protocol-specified AE reporting period, including signs or symptoms associated with the malignancy that were not present prior to the AE reporting period
- Complications that occur as a result of protocol-mandated interventions
- If applicable, AEs that occur prior to assignment of study treatment associated with medication washout, no treatment run-in, or other protocol-mandated intervention
- Preexisting medical conditions (other than the condition being studied) judged by the investigator to have worsened in severity or frequency or changed in character during the protocol-specified AE reporting period
- Diagnoses and/or symptoms associated with malignancy should be reported as AEs if they worsen or change in character. Clinical progression of malignancy should not be reported as an AE.

13.1.2 Serious Adverse Events

An AE should be classified as an SAE if:

- It results in death (i.e., the AE actually causes or leads to death).
- It is life threatening (i.e., the AE, in the view of the investigator, places the patient at immediate risk of death. It does not include an AE that, had it occurred in a more severe form, might have caused death.).
- It requires or prolongs inpatient hospitalization.
- It results in persistent or significant disability/incapacity (i.e., the AE results in substantial disruption of the patient's ability to conduct normal life functions).
- It is considered a significant medical event by the investigator based on medical judgment (e.g., may jeopardize the patient or may require medical/surgical intervention to prevent one of the outcomes listed above).

All AEs that do not meet any of the criteria for serious should be regarded as nonserious AEs.

The terms "severe" and "serious" are not synonymous. Severity (or intensity) refers to the grade of a specific AE, e.g., mild (Grade 1), moderate (Grade 2), or severe (Grade 3) myocardial infarction. "Serious" is a regulatory definition (see previous definition) and is based on patient or event outcome or action criteria usually associated with events that pose a threat to a patient's life or functioning. Seriousness (not severity) serves as the

guide for defining regulatory reporting obligations from the Sponsor to applicable regulatory authorities.

Severity and seriousness should be independently assessed when recording AEs and SAEs.

13.2 Methods and Timing for Assessing and Recording Safety Variables

The investigator is responsible for ensuring that all AEs and SAEs that are observed or reported during the study are recorded and reported to the sponsors (UNM CRTC) in accordance with protocol instructions. Since this protocol does not include an investigational agent, the SAE period will include the time of sample collection and 24 hours after the last collection at which time the port is de-accessed.

13.2.1 Assessment of Adverse Events

Investigators will assess the occurrence of AEs and SAEs at all patient evaluation time points during the study. All AEs and SAEs whether volunteered by the patient, discovered by study personnel during questioning, or detected through physical examination, laboratory test, or other means will be recorded in the patient's medical record.

Each recorded AE or SAE will be described by its duration (i.e., start and end dates), severity, regulatory seriousness criteria if applicable, suspected relationship to the investigational product (see following guidance), and actions taken.

The AE grading (severity) scale found in the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE), Version 3.0, will be used for AE reporting.

Table 3

Adverse Event Grading (Severity) Scale

Grade	*Severity*	*Alternate Description*
1	*Mild (apply event-specific NCI-CTCAE grading criteria)*	*Transient or mild discomfort (< 48 hours); no interference with the patient's daily activities; no medical intervention/therapy required*
2	*Moderate (apply event-specific NCI-CTCAE grading criteria)*	*Mild to moderate interference with the patient's daily activities; no or minimal medical intervention/therapy required*
3	*Severe (apply event-specific NCI-CTCAE grading criteria)*	*Considerable interference with the patient's daily activities; medical intervention/therapy required; hospitalization possible*
4	*Very severe, life threatening, or disabling (apply event-specific NCI-CTCAE grading criteria)*	*Extreme limitation in activity; significant medical intervention/therapy required, hospitalization probable*
5	*Death related to AE*	

Note: Regardless of severity, some events may also meet regulatory serious criteria. Refer to definitions of an SAE.
Use these alternative definitions for Grade 1, 2, 3, and 4 events when the

observed or reported AE is not in the NCI-CTCAE listing.

13.2.2 Independent Research Monitoring

The University of New Mexico Cancer Center (UNM CC) places a high priority on ensuring the safety of patients participating in clinical trials. All institutional trials require monitoring commensurate with the degree of risk, as identified by the Protocol Review & Monitoring Committee. The Independent Research Monitoring is the responsibility of the Data Safety Monitoring Committee, and for this protocol will be under the authority of the Vice-Chair of the DSMC is Elizabeth McGuire, MD, who is the independent research monitor. The UNM CC Independent Research monitoring is a process for routine real time data monitoring and subject safety review of Investigator Initiated trials in accordance with the Essential Elements of the National Cancer Institute Guidelines, the FDA monitoring regulations, Good Clinical Practice Guidelines and functions under the requirements of the UNM CC Data Safety Monitoring Plan (DSMP), as approved by the National Cancer Institute on September 26, 2011.

The duties and responsibilities of the Independent Research Monitoring Committee and Vice Chair include:

- Monitoring the progress of the trial and the safety of participants
- Monitoring of subject eligibility
- Monitoring of consent and data quality
- Monitoring of study conduct/compliance (accruals, treatment, toxicity and study outcome)
- Plans for assuring compliance with requirements regarding the reporting of adverse events (AEs)
- Plans for assuring data accuracy and protocol compliance, including minimization of risks.
- Determines whether the trial should be suspended or terminated based on ongoing reviews of safety data
- Has the authority to remove individual human subjects from the research protocol and take whatever steps is necessary to protect the safety and well-being of human subjects until the IRB assesses the Independent Research Monitor's report

The study will be monitored continuously for the first three patients of the trial (and reported to the PI no later than the time the third consecutive patient completes the study). After the third patient completes the study, the Independent Research Monitor will submit a study report to the Data Safety Monitoring Committee and the local Human Research Protections Office (HRPO). After the first oversight review, the Data Safety Monitoring Committee, Vice-Chair, Elizabeth McGuire, MD will designate the continued frequency with which the study will be monitored with no less than a quarterly review.

14.0 RESPONSIBILITIES OF THE PRINCIPAL INVESTIGATOR

The Principal Investigator is agreeing to comply with GCP and local regulations and guidelines as summarized below.

1. To conduct the study in accordance with the relevant, current protocol(s) and only make changes in a protocol after notifying the sponsor of the study (representatives of the Department of Defense), except when necessary to protect the safety, rights, or welfare of subjects.

2. To personally conduct or supervise the described investigation(s).

4. To report to adverse experiences that occur in the course of the investigation(s) in accordance with GCP and local law.

5. To read and understand the information in the study drug package insert, including the potential risks and side effects of the drug.

6. To ensure that all associates, colleagues, and employees assisting in the conduct of the study are informed about their obligations in meeting the above commitments

References

1. Parkin, D.M., Bray, F., Ferlay, J., Pisani, P. (2005) Global Cancer Statistics, 2002, CA Cancer J Clin 55:74-108.
2. Jemal, A., Siegel, R., Xu, J., Ward, E. (2010) Cancer Statistics, 2010, CA Cancer J Clin 5: 277-300.
3. Piccart, M. J., Bertelsen, K., Stuart, G., Cassidy, J., Mangioni, C., Simonsen, E., James, K., Kaye, S., Vergote, I., Blom, R., Grimshaw, R, Atkinson, R., Swenerton, K., Trope, C., Nardi, M., Kaern, J., Tumolo, S., Timmers, P., Roy, J. A., Lhoas, F, Lidvall, B., Bacon, M., Birt, A., Andersen, J., Zee, B., Paul, J., Pecorelli, S., Baron, B., McGuire, W. (2003) Long-term follow-up confirms a survival advantage of the paclitaxel-cisplatin regimen over the cyclophosphamide-cisplatin combination in advanced ovarian cancer, Int J Gynecol Cancer 13 Suppl 2:144-8.
4. Ozols, R.F., Rubin, S.C., Thomas, G., et al. Epithelial ovarian cancer, in Hoskins, W.Y., Perez, C.A., Young, R.C. (eds). (2005) Principles and Practice of Gynecologic Oncology, (ed 4) Philadelphia, PA, Lippincott Williams & Wilkins, 919-922.
5. Vergote, I., Trope, C. G., Amant, F., Kristensen, G. B., Ehlen, T., Johnson, N., Verheijen, R. H., van der Burg, M. E., Lacave, A. J., Panici, P. B., Kenter, G. G., Casado, A., Mendiola, C., Coens, C., Verleye, L., Stuart, G. C., Pecorelli, S., Reed, N. S. (2010) Neoadjuvant chemotherapy or primary surgery in stage IIIC or IV ovarian cancer, N Engl J Med 10:943-53.
6. Runowicz, C. D. (2008) Intraperitoneal chemotherapy in ovarian cancer: an update, Cancer J 1:7-9.
7. Trimble, E. L., Christian, M. C. (2008) National Cancer Institute-United States strategy regarding intraperitoneal chemotherapy for ovarian cancer, Int J Gynecol Cancer 18 Suppl 1: 26-8.
8. Fujiwara, K., Aotani, E., Hamano, T., Nagao, S., Yoshikawa, H., Sugiyama, T., Kigawa, J., Aoki, D., Katsumata, N., Takeuchi, M., Suzuki, M. (2011) A randomized Phase II/III trial of 3 weekly intraperitoneal versus intravenous carboplatin in combination with intravenous weekly dose-dense paclitaxel for newly diagnosed ovarian, fallopian tube and primary peritoneal cancer, Jpn J Clin Oncol 2:278-82
9. Barrena Medel, N. I., Wright, J. D., Herzog, T. J. (2010) Targeted therapies in epithelial ovarian cancer, J Oncol 2010:314326.
10. Burger, R. A., Sill, M. W., Monk, B. J., Greer, B. E., Sorosky, J. I. (2007) Phase II trial of bevacizumab in persistent or recurrent epithelial ovarian cancer or primary peritoneal cancer: a Gynecologic Oncology Group Study, J Clin Oncol 33: 5165-71.
11. Cannistra, S. A., Matulonis, U. A., Penson, R. T., Hambleton, J, Dupont, J., Mackey, H., Douglas, J., Burger, R. A., Armstrong, D., Wenham, R., McGuire, W. (2007) Phase II study of bevacizumab in patients with platinum-resistant ovarian cancer or peritoneal serous cancer, J Clin Oncol 33:5180-6.
12. Garcia, A. A., Hirte, H., Fleming, G., Yang, D., Tsao-Wei, D. D., Roman, L., Groshen, S., Swenson, S., Markland, F., Gandara, D., Scudder, S., Morgan, R., Chen, H., Lenz, H. J., Oza, A. M. (2008) Phase II clinical trial of bevacizumab and low-dose metronomic oral cyclophosphamide in recurrent ovarian cancer: a trial of the California, Chicago, and Princess Margaret Hospital phase II consortia, J Clin Oncol 26:76-82.

13. Tillmanns, T.D., Lowe, M.P., Schwartzberg, L.S., Walker, M.S., Stepanski, E.J. (2010) A phase II study of bevacizumab with nab-paclitaxel in patients with recurrent, platinumresistant primary epithelial ovarian or primary peritoneal carcinoma, J Clin Oncol 28, 15 suppl, Abstract 5009.
14. Penson, R. T., Dizon, D. S., Cannistra, S. A., Roche, M. R., Krasner, C. N., Berlin, S. T., Horowitz, N. S., Disilvestro, P. A., Matulonis, U. A., Lee, H., King, M. A., Campos, S. M. (2010) Phase II study of carboplatin, paclitaxel, and bevacizumab with maintenance bevacizumab as first-line chemotherapy for advanced mullerian tumors, J Clin Oncol 1: 154-9.
15. Burger, R.A., Brady, M.F., Bookman, J.L., Walker, H.D., Homesley, J., Fowler, B.J., Monk, B.E., Greer, M., Liang, S.X. (2010) Phase III trial of bevacizumab (BEV) in the primary treatment of advanced epithelial ovarian cancer (EOC), primary peritoneal cancer (PPC), or fallopian tube cancer (FTC): A Gynecologic Oncology Group study, J Clin Oncol 28, 18 suppl, abstr LBA1.
16. Kristensen, T., Perrem, T., Qian, W., Pfisterer, J., Ledermann, A., Joly, F., Carey, M.S., Beale, P.J., Cervantes, A., Oza, A.M. (2011) Results of interim analysis of overall survival in the GCIG ICON7 phase III randomized trial of bevacizumab in women with newly diagnosed ovarian cancer, J Clin Oncol 29: 18 suppl, abstr LBA5006.
17. Aghajanian, C., Finkler, N.J., Rutherford, T., Smith, D.A., Yi, J., Parmar, H., Nycum, L.R., Sovak, M.A. (2011) OCEANS: A randomized, double-blinded, placebo controlled phase III trial of chemotherapy with or without bevacizumab (BEV) in patients with platinum-sensitive recurrent epithelial ovarian (EOC), primary peritoneal (PPC), or fallopian tube cancer (FTC), J Clin Oncology 29, 18 suppl, abstr LBA5007.
18. Cohn, D. E., Kim, K. H., Resnick, K. E., O'Malley, D. M., Straughn, J. M., Jr. (2011) At what cost does a potential survival advantage of bevacizumab make sense for the primary treatment of ovarian cancer? A cost-effectiveness analysis, J Clin Oncol 29: 1247-51.
19. Surviladze, Z., Waller, A., Wu, Y., Romero, E., Edwards, B. S., Wandinger-Ness, A., and Sklar, L. A. (2010) Identification of a small GTPase inhibitor using a high-throughput flow cytometry bead-based multiplex assay, J Biomol Screen 15:10-20.
20. Agola, J. O., Surviladze, Z., Buranda, T., Ursu, O., Hong, L., Waller, A., Strouse, J. J., Simpson, D. S., Schroeder, C. E., Golden, J. E., Oprea, T. I., Sklar, L. A., and Wandinger-Ness, A. (2011) A Competitive Nucleotide Binding Inhibitor: In vitro Characterization of Rab7 GTPase Inhibition, ACS Chemical Biology (Submitted)
21. Jaffe, A. B., and Hall, A. (2005) Rho GTPases: biochemistry and biology, Annu Rev Cell Dev Biol 21:247-269.
22. Karlsson, R., Pedersen, E. D., Wang, Z., and Brakebusch, C. (2009) Rho GTPase function in tumorigenesis, Biochim Biophys Acta 1796: 91-98.
23. Ridley, A. J. (2006) Rho GTPases and actin dynamics in membrane protrusions and vesicle trafficking, Trends Cell Biol 16: 522-529.
24. Vega, F. M., and Ridley, A. J. (2008) Rho GTPases in cancer cell biology, FEBS Lett 582:2093-2101.

25. Wennerberg, K., Rossman, K. L., and Der, C. J. (2005) The Ras superfamily at a glance, J Cell Sci 118: 843-846.

26. Fritz, G., and Kaina, B. (2006) Rho GTPases: promising cellular targets for novel anticancer drugs, Curr Cancer Drug Targets 6:1-14.

27. Ferri, N., Corsini, A., Bottino, P., Clerici, F., and Contini, A. (2009) Virtual screening approach for the identification of new Rac1 inhibitors, J Med Chem 52:4087-4090.

28. Nassar, N., Cancelas, J., Zheng, J., Williams, D. A., and Zheng, Y. (2006) Structure-function based design of small molecule inhibitors targeting Rho family GTPases, Curr Top Med Chem 6: 1109-1116.

29. Cohen, A., and Basch, C. (1988) Steady state pharmacokinetics of naproxen in young and elderly healthy volunteers, Semin Arthritis Rheum 17: 7-11.

30. Furst, D. E., Sarkissian, E., Blocka, K., Cassell, S., Dromgoole, S., Harris, E. R., Hirschberg, J. M., Josephson, N., and Paulus, H. E. (1987) Serum concentrations of salicylate and naproxen during concurrent therapy in patients with rheumatoid arthritis, Arthritis Rheum 30:1157-1161.

31. Handley, D. A., Cervoni, P., McCray, J. E., and McCullough, J. R. (1998) Preclinical enantioselective pharmacology of (R)- and (S)- ketorolac, J Clin Pharmacol 38:25S-35S.

32. Jett, M. F., Ramesha, C. S., Brown, C. D., Chiu, S., Emmett, C., Voronin, T., Sun, T., O'Yang, C., Hunter, J. C., Eglen, R. M., and Johnson, R. M. (1999) Characterization of the analgesic and anti-inflammatory activities of ketorolac and its enantiomers in the rat, J Pharmacol Exp Ther 288:1288-1297.

33. Buckley, M. M., and Brogden, R. N. (1990) Ketorolac. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic potential, Drugs 39:86-109.

34. Mroszczak, E. J., Jung, D., Yee, J., Bynum, L., Sevelius, H., and Massey, I. (1990) Ketorolac tromethamine pharmacokinetics and metabolism after intravenous, intramuscular, and oral administration in humans and animals, Pharmacotherapy 10: 33S-39S.

35. Pallapies, D., Peskar, B. A., Brune, K., and Geisslinger, G. (1994) Effects on platelet functions and pharmacokinetics of azapropazone and ketorolac tromethamine given as single parenteral doses, Br J Clin Pharmacol 37:335-339.

36. Pallapies, D., Salinger, A., Meyer zum Gottesberge, A., Atkins, D. J., Rohleder, G., Nagyivanyi, P., and Peskar, B. A. (1995) Effects of lysine clonixinate and ketorolac tromethamine on prostanoid release from various rat organs incubated ex vivo, Life Sci 57: 83-89.

37. Kean, W. F., Lock, C. J., Rischke, J., Butt, R., Buchanan, W. W., and Howard-Lock, H. (1989) Effect of R and S enantiomers of naproxen on aggregation and thromboxane production in human platelets, J Pharm Sci 78:324-327.

38. Hinz, B., Cheremina, O., Besz, D., Zlotnick, S., and Brune, K. (2008) Impact of naproxen sodium at over-the-counter doses on cyclooxygenase isoforms in human volunteers, Int J Clin Pharmacol Ther 46:180-186.

39. Huntjens, D. R., Spalding, D. J., Danhof, M., and Della Pasqua, O. E. (2006) Correlation between in vitro and in vivo concentration-effect relationships of naproxen in rats and healthy volunteers, Br J Pharmacol 148:396-404.

40. Kean, W. F., Lock, C. J., and Howard-Lock, H. E. (1991) Chirality in antirheumatic drugs, Lancet 338:1565-1568.

41. Mroszczak, E., Combs, D., Chaplin, M., Tsina, I., Tarnowski, T., Rocha, C., Tam, Y., Boyd, A., Young, J., and Depass, L. (1996) Chiral kinetics and dynamics of ketorolac, J Clin Pharmacol 36:521-539.

42. Fisher, K. E., Sacharidou, A., Stratman, A. N., Mayo, A. M., Fisher, S. B., Mahan, R. D., Davis, M. J., and Davis, G. E. (2009) MT1-MMP- and Cdc42-dependent signaling co-regulate cell invasion and tunnel formation in 3D collagen matrices, J Cell Sci 122: 4558-4569.

43. Machesky, L.M. (2008) Lamellipodia and filopodia in metastasis and invasion, FEBS Lett 582:2102-2111.

44. Lynn Am, Bradford H, Kantor ED, Kok-Yong S, Salinger DH, Chen J, Ellenbogen RG, Vicini P, Anderson GD. (2007) Postoperative ketorolac tromethaminine use in infants aged 6-18 months: the effect of morphine usage, safety assessment and stero-specific pharmacokinentics. Anesthesia and Analgesia 104:1040-1051.

45. Gotlieb WH, Bruchim I, Ben-Baruch G, Davidson B, Zeltser A, Andersen A, Olsen H. (2007) Doxorubicin levels in serum and ascites of patients with ovarian cancer. EJSO 33:213-215.

46. Ning Y, Zeineldin R, Liu Y, Rosenberg M, Stack MS, Hudson LG. (2005) Down-regulation of integrin alpha2 surface expression by mutant epidermal growth factor receptor (EGFRviii) induces aberrant cell spreading and focal adhesion formation. Cancer Rse 65:9280-9286.