

Supporting information for:

**Auranofin-Based Analogues Are Effective Against Clear Cell Renal
Carcinoma *in vivo* and Display No Significant Systemic Toxicity**

Benelita T. Elie,^{a,b} Karen Hubbard,^{b,e} Buddhadev Layek,^f Won Seok Yang,^g Swayam Prabha,^f Joe W. Ramos,^{g,} and Maria Contel^{a,b-d,g,*}*

^aDepartment of Chemistry, Brooklyn College, The City University of New York; Brooklyn, New York; ^bBiology, ^cChemistry and ^dBiochemistry PhD Programs, The Graduate Center, The City University of New York, New York, New York; ^eDepartment of Biology, City College of New York, The City University of New York, New York, New York; ^fUniversity of Minnesota, College of Pharmacy, Minneapolis, Minnesota; ^gUniversity of Hawaii Cancer Center, Honolulu, Hawaii.

This PDF includes Supplementary Tables 1, 2 and 3 related to the pathology study.

Table S1. Summary of plasma metabolic analytes for specimens from tumor bearing NOD-SCID (NOD/LtSz-Prkdcid/j) mice. The study criteria reflect pathological criterion used in clinical studies. Metabolic markers were quantified from freshly isolated plasma and compared to reference range. Titanocref: 3 specimens, at 5 mg/kg/72 hours over 21 days. Titanofin: 2 specimens, at 10mg/kg/72 hours over 21 days.

	Titanocref	Titanofin	Vehicle	Reference Ranges
BUN (mg/dL)	22.33	21.50	21.50	5.0-28
CREA (mg/dL)	0.17	0.17	0.16	0.2-0.5
BUN/CREA ratio	128.99*▼	126.47*▼	142.50	-
ALP (U/L)	37*▼	42.5*▼	55.50	105-370
ALT (U/L)	36.33*▼	167*▼	199.00	27-195
AST (U/L)	135.67*▼◎	374.5*▼◎	402.50◎	54-77
GGT (U/L)	0.00	0.00	0.00	-
TBIL (mg/dL)	0.17	0.20	0.20	0.2-0.6
DBIL (mg/dL)	0.03	0.05	0.00	-
IBIL (mg/dL)	0.13	0.15	0.20	-
TP (g/dL)	5.03	5.3*▲	4.95	4.8-7.2
ALB (g/dL)	2.70	2.95	2.80	2.4-4.3
GLOB (g/dL)	2.33	2.35	2.15	1.7-2.2
A/G ratio	1.17	1.26	1.30	-
P (mg/dL)	8.87	9.95	9.80	7.3-14.5
Ca (mg/dL)	9.33	9.60	9.20	9.5-12.5
GLU (mg/dL)	166.33*▼	170*▼	189.50	172-372
CHOL (mg/dL)	85.00	88.00	85.50	55-169
TRIG (mg/dL)	109*▲	76.50	74.00	67-289
CK (U/L)	278.67*▼◎	196.5*▼◎	566.50	428-1609
TCO2 (mEq/L)	22.67	21.50	18.00	-
Na (mEq/L)	157.00	155.00	156.00	145-181
K (mEq/L)	8.13	9.30	10.00	7.3-11.1
Cl (mEq/L)	110.67	112.00	115.00	111-134
Na/K ratio	19.66	16.74	16.24	-
Anion Gap	31.80	30.8*▼	33.00	-

* P < 0.05, values statistically different between Vehicle-treated and Titanocref-treated or Titanofin-treated groups. ▼ Titanocref or Titanofin induce a decrease from Veh treated group, ▲ Titanocref or Titanofin induce an increase from Veh treated group, ◎ deviates significantly from the normal range.

ALP, alakaline phosphatase; AST, aspartate aminotransferase; Ca, calcium; Cl, chloride; CPK, creatine phosphokinase; P, phosphorus; K, potassium; Na, sodium; BUN, blood urea nitrogen; Statistical significance (*P <0.05).

Table S2. Summary of hematology values (mean) for specimens from NOD-SCID (NOD/LtSz-Prkdcid/j) mice. Complete blood counts of each group. Heparinized blood acquired from mice was assessed using the automatic Hematology Analyzer. Blood smears were carried out and fixed with methanol, stained, and observed under the microscope to carry out differential blood white cell counts. Titanocref: 3 specimens, at 5 mg/kg/72 hours over 21 days. Titanofin: 2 specimens, at 10mg/kg/72 hours over 21 days.

Automatic count

	Titanocref	Titanofin	Vehicle	NSG Reference Range
RBC (M/uL)	9.02*▲	9.375*▲	7.415	7.84-10.84
HGB (g/dL)	13.83*▲	14.3*▲	11.45	11.8-17.6
HCT (%)	45.67*▲	48.35*▲	36.9	44.1-58.3
MCV (fL)	50.67	51.55	49.1	51.1-58.6
MCH (pg)	15.33	15.25	15.5	13.7-17.2
MCHC (g/dL)	30.30	29.6	31.7	25.1-31.3
RDW-SD (fL)	29.23	30.65	29.35	-
RDW-CV (%)	22.23	22.5	20.5	17.3-20.3
RET# (K/uL)	318.47*▼	427.55	444.4	294-444
RET (%)	3.52	4.56	4.62	2.56-4.56
PLT (K/uL)	1244.00*▲	1327.5*▲	712.5	651-2055
PDW (fL)	6.67	6.65	6.5	-
MPV (fL)	6.1	6.05	5.9	4.2-6.3
Morphology	1+ Echinocytes. 3+ polychromasia. 2+ HGB Crystals. 2+ polychromasia. 3+ PLT clumps.	2+ polychromasia. A manual differential was not performed due to low WBC count (<2.00 K/uL).	2+ polychromasia.	

**Manual
differential**

	Titanocref	Titanofin	Vehicle	NSG Reference Range
Neut# (K/uL)	2.21*▲	0.845*▼	1.675	0.54-3.16
Band# (K/uL)	0.00	0	0.01	-
LYMPH# (K/uL)	0.85*▲	0.38	0.41	0.23-1.56
MONO# (K/uL)	0.00	0	0	0.03-0.26
EO# (K/uL)	0.02*▼	0.125*▲	0.075	0.00-0.39
NEUT (%)	68.33	62*▼	75.5	44.21-79.92
LYMPH (%)	26.33*▲	28*▲	18.5	13.51-42.61
MONO (%)	4.50*▲	5*▲	2.5	1.71-10.93
EO (%)	3.50	5*▲	3	0.29-10.32
Morphology	WBC morphology is within normal limits.	WBC morphology is within normal limits.	WBC morphology is within normal limits.	

* P < 0.05, values statistically different between Vehicle-treated and Titanocref-treated or Titanofin-treated groups. ▼ Titanocref or Titanofin induce a decrease from Veh treated group, ▲ Titanocref or Titanofin induce an increase from Veh treated group.

WBC: white blood cells, RBC: red blood cells, Hgb: hemoglobin, HCT: hematocrit, MCV: mean cell volume, MCH: mean corpuscular hemoglobin content, MCHC: mean corpuscular hemoglobin concentration, RDW-CV: red cell distribution width-coefficient of variation, PLT: platelets, MPV: mean platelet volume.

Table S3. Histology. Summary of Histopathological Findings.

	Titanocref	Titanofin	Vehicle
Body weight (g)	26.078	20.887	21.306
Liver weight (g)	1.363	1.147	0.932
Spleen weight (g)	0.040	0.048	0.050
Heart weight (g)	0.203	0.148	0.120
Left Kidney weight (g)	0.225	0.199	0.144
Right Kidney weight (g)	0.250	0.168	0.147
Heart	N	N	N
Lungs	N	Alveolar histiocytosis, MF, 2.	Alveolar histiocytosis, MF, 1.
Thymus	U	N	N
Kidneys	N	N	N
Liver	N	N	N
Gallbladder	N	N	N
Stomach	N	N	N
Duodenum, jejunum, ileum	N	N	N
Cecum	Lymphoplasmacytic and neutrophilic aggregates, submucosal, F, 2.	N	Lymphoplasmacytic and neutrophilic aggregates, submucosal, F, 2.
Colon	N	N	N
Mesenteric lymph node	N	N	N
Salivary glands	N	N	N
Submandibular lymph node	N	N	N
Uterus	N	N	Cystic endometrial hyperplasia, MF, 2.
Cervix	N	N	N
Vagina	N	N	N
Testes/epididymis	Testicular interstitial edema, BL, D, 2.	N	N
Prostate	N	N	N
Seminal vesicles	N	N	N
Urinary bladder	N	N	N
Spleen	Increased extramedullary hematopoiesis, 2	Increased extramedullary hematopoiesis, 2	Increased extramedullary hematopoiesis, 2
Pancreas	N	N	Interstitial edema, D, 2.

Adrenals	N	N	N
Ovaries	N	N	N
Oviducts	N	N	N
Trachea	N	N	N
Esophagus	N	N	N
Thyroid	N	N	N
Parathyroid	U	U	U
Skin (trunk)	Mass 1 and 2: Necrotic debris and suppurative inflammation, with eosinophilic flaky birefringent material and fibrovascular stroma, F, 4	Mass 1: Anaplastic neoplasm; Mass 2: Necrotic debris with eosinophilic flaky birefringent material, 2	Mass 1 and 2: Multifocal anaplastic neoplasm; Mass 3: Eosinophilic flaky birefringent material with rare necrotic debris, F, 2
Mammary glands	NA	N	N
Bones (femur, tibia, sternum, vertebrae)	N	N	N
Bone marrow (femur, tibia, sternum, vertebrae)	Granulocytic hyperplasia, D, 2; Erythroid hypoplasia; D, 2.	N	N
Stifle joint	N	N	N
Skeletal muscles (hind limb, spine)	N	N	N
Nerves (hind limb, spine)	N	N	N
Spinal cord	N	N	N
Oral cavity	N	N	N
Teeth	N	N	N
Nasal cavity	N	N	N
Eyes	N	N	N
Harderian gland	N	N	N
Bones (skull)	N	N	N
Pituitary	N	N	N
Brain	N	N	N
Ears	N	N	N
Other organs	N	N	N

N: Normal

F: Focal

MF: Multifocal

D: Diffuse

BL: Bilateral

1: Minimal

2: Mild

3: Moderate

4: Marked

U: Unavailable