**SUPPLEMENTARY TABLES**

**Table S1. Representative clinical trials in high-grade gliomas, brain metastases, and other solid tumors treated with nanoformulated or free doxorubicin and other selected therapies**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Identifier** | **Title** | **Phase/****Status** | **Rationale/Goals** | **Location** |
| NCT00795678 | Clinical Study to Assess Entry of Chemotherapeutic Agents into Brain Metastases in Women with Breast Cancer  | P1(C) | Assess target tissue penetration, inform future rational drug designs and regimens | MSK, Cleveland Clinic |
| NCT01702129 | Clinical Study of DOX Loaded Anti-EGFR Immunoliposomes in Patients with Advanced Solid Tumors | P1(C) | Antibody-coated immunoliposomes attach selectivelyto antigens expressed on target cells and are internalized efficiently. | University Hospital, Basel, Switzerland |
| NCT00011414 | Pharmacokinetic Study of Tariquidar (a P-Glycoprotein Inhibitor) in Combination with DOX, Vinorelbine or Docetaxel in Pediatric Patients with Refractory Solid Tumors, Including Brain Tumors | P1(C) | Evaluate tolerance and effects of tariquidar, combined with other anticancer drugs (DOX, vinorelbine, docetaxel) for treating solid tumors.  | NIHClinical Center |
| NCT00019630 | Study of DOX HCl Liposome in Pediatric Patients with Refractory Solid Tumors | P1(C) | Determine tolerance, toxicity of DOX HCl liposome, MTD, PK, and whether serum troponin T is a useful biomarker of DOX-induced myocardial damage. | Children’sHospital of Philadelphia,Pediatric Oncology |
| NCT01386580 | An Open-label, Dose Escalating Study of 2B3-101 in Patients with Solid Tumors and Brain Metastases or Recurrent Malignant Glioma | P1/2a(C) | Determine safety, tolerability and PK of glutathione PEG- liposomal DOX as a single agent or combined with trastuzumab. Assess antitumor activity as a single agent in brain metastases or recurrent malignant glioma or combined with trastuzumab in HER2+ breast cancer with brain metastases. | United States, Belgium, France, Netherlands(multiple locations) |
| NCT00002608 | Cisplatin, DOX, and Tamoxifen in the Treatment of Incurable Soft Tissue and Endocrine Malignancies  | P2(C) | Assess effectiveness of cisplatin and DOX together with tamoxifen in solid tumors | Ottawa Regional Cancer Ctr, Ottawa Hosp, Canada, Ontario |
| NCT02766699 | Study to Evaluate the Safety, Tolerability, and Immunogenicity of EGFR (Vectibix® Sequence)-Targeted EnGeneIC Dream Vectors Containing DOX (EGFR(V)-EDV-DOX) in Subjects with Recurrent GBM | P1(R) | Assess safety, tolerability, dosing of EGFR(V)-EDV-DOX; immune responses, effectiveness in recurrent GBM. (n.b. EGFR(V)-EDV-DOX uses EnGeneIC EDV, bacterial-derived minicells packaging DOX in 400 nm particles adapted with bispecific antibodies) | John Hopkins Hospital; Lenox Hill Hospital |
| NCT02343991 | A Study to Evaluate the Safety and Feasibility of Blood-Brain Barrier Disruption Using Transcranial MRI-Guided Focused Ultrasound with Intravenous Ultrasound Contrast Agents in the Treatment of Brain Tumors with DOX | P1, active, (NR) | Assess safety, BBB disruption using transcranial MRI-guided focused U/S and an IV U/S contrast agent to increase uptake of DOX in brain tumors and adjacent brain. Data will inform efficacy studies. | Sunnybrook Health Sciences CtrCanada, Ontario |
| NCT02372409 | A Pilot Study Using MRI-Guided Laser Heat Ablation to Induce Disruption of the Peritumoral Blood-Brain Barrier to Enhance Delivery and Efficacy of Treatment of Pediatric Brain Tumors | P1(R) | Correlate advanced MRI with serum biomarkers of BBB disruption to identify the window of maximal BBB disruption post MRI-guided laser ablation to allow for alternatives to surgery in incompletely resected tumors, improving benefits and risks using optimal chemotherapy doses and distinguishing tumor progression from long-term effects of laser treatment.  | Washington University School of Medicine |
| NCT02735798 | A Pilot Study of 64Cu-labeled Brain PET/MRI for MM-302, a Novel HER2-Targeting Agent in Advanced HER2+ Cancer with Brain Metastases | P1(W, not funded) | Assess new and progressive HER2+ cancers with brain metastases using PET-MRI imaging, 64Cu-MM-302 (HER2-targeted antibody–liposomal DOX conjugate) and unlabeled MM-302 combined with trastuzumab  | No Contacts or Locations Provided |
| NCT00465673 | Study of Liposomal Doxorubicin (Lipo-Dox) in Patients with Brain Metastasis from Breast Cancer | P2(T, low recruit) | Assess treatment response rate of Lipo-Dox in breast cancer patients with brain metastasis | Johns Hopkins Singapore International Medical Center, Singapore |

***Legend:*** *C, completed; R, recruiting; NR, not recruiting; W, withdrawn; T, terminated; DOX, doxorubicin; ma; BCBM, breast cancer brain metastases; GBM, glioblastoma multiforme; PK, pharmacokinetics; MTD, maximum tolerated dose; BBB, blood-brain barrier; U/S, Ultrasound*

**Table S2. The cumulative clearance of 89Zr-DFO-DOX-C’ dots in mouse urine and feces at different post-injection time points (n=3). Note, the radioactivity in both urine and feces was measured using a CRC®-55tR Dose Calibrator and presented as %ID (mean ± SD).**

|  |  |  |
| --- | --- | --- |
|  | **Urine (%ID)** | **Feces (%ID)** |
|  | Ave. | SD | Ave. | SD |
| *6 h* | 13.9 | 1.2 | 1.0 | 1.5 |
| *24 h* | 28.4 | 0.7 | 8.8 | 1.3 |
| *48 h* | 38.3 | 1.2 | 19.0 | 6.3 |
| *72 h* | 41.1 | 2.7 | 20.5 | 7.6 |
| *120 h* | 44.7 | 2.5 | 21.7 | 7.9 |
| *168 h* | 47.3 | 2.7 | 22.2 | 8.2 |

**Table S3. *Ex vivo* biodistribution data of 89Zr-DFO-DOX-C’ dots at 168 h (on Day 7) post-injection (n=3, from a metabolic cage study). Radioactivity in major organs and tissues was measured using a gamma-counter and presented as %ID (mean ± SD).**

|  |  |  |
| --- | --- | --- |
| **Organ (%ID)** | **Ave.** | **SD** |
| Blood | 0.2 | 0.1 |
| Heart | 0.4 | 0.1 |
| Lungs | 0.3 | 0.1 |
| Liver | 3.7 | 0.8 |
| Spleen | 0.2 | 0.1 |
| Stomach | 0.1 | 0.1 |
| Sm. Int. | 1.1 | 0.4 |
| Lg. Int. | 0.5 | 0.1 |
| Kidneys | 0.5 | 0.1 |
| Brain | 0.01 | 0.00 |
| Carcass | 30.1 | 0.7 |
| Feces | 22.2 | 8.2 |
| Urine | 47.3 | 2.7 |

**Table S4. Radiation dosimetry of 89Zr-DFO-DOX-C’ dots (DPR=30) in a 70-kg standard man estimated by using the OLINDA dosimetry program.**



n.b. Mouse absorbed doses were calculated assuming complete local

 absorption of the particulate radiations only.

**Table S5. *Ex vivo* biodistribution data of 89Zr-DFO-DOX-C’ dots at 48- and 72-h post-injection (n=3 mice/time point) in PDGF-B glioma mice. Radioactivity in major organs and tissues were measured using a gamma counter and presented as %ID/g (mean ± SD).**

|  |  |  |
| --- | --- | --- |
| ***%ID/g*** | **48 h** | **72 h** |
| ***Ave.*** | ***SD*** | ***Ave.*** | ***SD*** |
| **Blood** | 3.9 | 0.2 | 2.8 | 0.5 |
| **Tumor** | 3.0 | 0.2 | 3.3 | 0.1 |
| **Heart** | 3.4 | 0.5 | 3.5 | 0.3 |
| **Lungs** | 3.5 | 0.3 | 2.9 | 0.6 |
| **Liver** | 4.2 | 0.3 | 4.2 | 0.1 |
| **Spleen** | 3.3 | 0.2 | 2.6 | 0.4 |
| **Stomach** | 1.7 | 0.3 | 1.3 | 0.3 |
| **Sm. Int.** | 2.3 | 0.4 | 2.1 | 0.9 |
| **Lg. Int.** | 1.2 | 0.1 | 1.4 | 0.1 |
| **Feces** | 0.9 | 0.1 | 0.7 | 0.1 |
| **Kidneys** | 3.3 | 0.2 | 1.6 | 0.3 |
| **Muscle** | 0.6 | 0.2 | 1.3 | 0.2 |
| **Bone** | 1.6 | 0.3 | 1.6 | 0.1 |

**Table S6.** Triple-dose toxicity testing in tumor-bearing mice after systemic administration of saline vehicle (**Control group**, *n*=3), DOX alone (**Treatment group #1**, 7 mg/kg/dose, cumulative dose 21 mg/kg, *n*=3), or Dox-NDC (**Treatment group #2**, 7 mg/kg/dose, cumulative dose 21 mg/kg, *n*=3)

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Test Name** | ***#1 Saline*** | ***#2 Saline*** | ***#3 Saline*** | ***#4******Saline*** | ***#1******Dox*** | ***#2******Dox*** | ***#3******Dox*** | ***#4******Dox*** | ***#5******Dox*** |
| **Gender** | Male | Male | Female | Female | Female | Male | Female | Female | Female |
| **Sacrifice Day** | *7* | *7* | *7* | *7* | *7* | *7* | *7* | *13* | *13* |
|  |  |  |  |  |  |  |  |  |  |
| **Gross Examination** |  |  |  |  |  |  |  |  |  |
| **Body weight (g)** | 21.5 | 19.23 | 20.71 | 21.08 | 17.06 | 16.57 | 17.95 | 13.11 | 14.19 |
| **Heart weight (g)** | 0.120 | 0.128 | 0.128 | 0.144 | 0.104 | 0.130 | 0.109 | 0.083 | 0.095 |
| **Histologic examination** | 9.80 | 8.10 | 5.40 | 8.90 |  |  |  |  |  |
| **Heart TUNEL** | N | N | N | N | N | N | N | N | N |
| **Heart Cleaved caspase-3** | N | N | N | N | N | Left/right atrium: Positive myocardial cells, MF, 2. | Left/right atrium: Positive myocardial cells, MF, 2. | N | N |
|  |  |  |  |  |  |  |  |  |  |

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Test Name** | ***#6*** ***Dox*** | ***#7*** ***Dox*** | ***#8*** ***Dox*** | ***#9******Dox*** | ***#1******Dox-C’ dots*** | ***#2******Dox-******C’ dots*** | ***#3******Dox-C’ dots*** | ***#4******Dox-C’ dots*** | ***#5******Dox-C’ dots*** |
| **Gender** | Male | Male | Female | Female | Female | Male | Female | Female | Female |
| **Sacrifice Day** | *13* | *13* | *20* | *20* | *9* | *9* | *15* | *15* | *15* |
|  |  |  |  |  |  |  |  |  |  |
| **Gross Examination** |  |  |  |  |  |  |  |  |  |
| **Body weight (g)** | 12.88 | 13.90 | 18.03 | 21.33 | 22.46 | 23.12 | 21.60 | 21.20 | 25.07 |
| **Heart weight (g)** | 0.092 | 0.087 | 0.119 | 0.129 | 0.122 | 0.129 | 0.136 | 0.126 | 0.136 |
| **Histologic examination** | 9.80 | 8.10 | 5.40 | 8.90 |  |  |  |  |  |
| **Heart TUNEL** | N | N | N | N | NE | NE | NE | NE | NE |
| **Heart Cleaved caspase-3** | Left ventricular wall: Positive myocardial cells, MF, 2. | Left atrium/ ventricular wall: Positive myocardial cells, MF, 1. | N | Left/right atrium: Positive myocardial cells, MF, 1. | NE | NE | NE | NE | NE |
|  |  |  |  |  |  |  |  |  |  |

***Legend:*** *N: Normal; MF: Multifocal; NE: not evaluated*

**Table S7. Evaluation of Hematological Parameters**

|  |  |
| --- | --- |
| Red Blood Cell Count (RBC) | Platelet Count (PLT) |
| Hemoglobin (HGB) | Platelet distribution width (PDW) |
| Hematocrit (HCT) | Mean Platelet Volume (MPV)  |
| Mean Corpuscular Volume (MCV) | White Blood Cell Count (WBC) |
| Mean Corpuscular Hemoglobin (MCH) | Neutrophil Count (NEUT) |
| Mean Corpuscular Hemoglobin Concentration (MCHC) | Lymphocyte Count (LYMPH) |
| Red Blood Cell Distribution Width-standard deviation (RDW-SD) | Monocyte Count (MONO) |
| Red Blood Cell Distribution Width-standard deviation-CV | Eosinophil Count (EO) |
| Reticulocyte Number per Volume (RET#) | Basophil Count (BASO) |
| Percent Reticulocytes (RET %) | Neutrophil Percentage (NEUT %) |

**Table S8.** Complete blood counts of mice injected with saline vehicle (**Control group**, *n*=3),DOX alone(**Treatment group #1**,7 mg/kg/dose,cumulative dose 21 mg/kg, *n*=3), or DOX-C’ dots (**Treatment group #2**,7 mg/kg/dose, cumulative dose 21 mg/kg, *n*=3)

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Test Name** |  **Reference** | ***#1 Saline*** | ***#2 Saline*** | ***#3 Saline*** | ***#1******DOX*** | ***#2******DOX*** | ***#3******DOX*** | ***#1******DOX-C’ dots*** | ***#2******DOX-C’ dots*** | ***#3******Dox-C’ dots*** |
| **Gender** |  | Male | Male | Female | Female | Female | Male | Female | Female | Female |
| **Sacrifice Day** |  | *9* | *9* | *9* | *10* | *10* | *15* | *12* | *15* | *15* |
|  |  |  |  |  |  |  |  |  |  |  |
| **RBC (M/uL)** | *7.14-12.20* | 8.85 | 9.76 | 8.54 | 7.37 | 8.08 | 8.78 | 8.68 | 8.55 | 8.01 |
| **HGB (g/dL)** | *10.8-19.2* | 13.8 | 15.8 | 14.3 | 11.6 | 13.0 | 13.40 | 14.3 | 14.30 | 13.60 |
| **HCT (%)** | *37.2-62.0* | 46.4 | 52.6 | 47.6 | 38 | 41.1 | 44.10 | 44.1 | 48.00 | 47.00 |
| **MCV (fL)** | *42.6-56.0* | 52.4 | 53.9 | 55.7 | 51.6 | 50.9 | 50.20 | 50.8 | 56.10 | 58.70 |
| **MCH (pg)** | *11.7-16.8* | 15.6 | 16.2 | 16.7 | 15.7 | 16.1 | 15.30 | 16.5 | 16.70 | 17.00 |
| **MCHC (g/dL)** | *24.6-35.9* | 29.7 | 30 | 30 | 30.5 | 31.6 | 30.40 | 32.4 | 29.80 | 28.90 |
| **RDW-SD (fL)** | *--* | 29.3 | 31.9 | 31.7 | 28.1 | 27.3 | 28.40 | 27.3 | 35.90 | 39.50 |
| **RDW-CV (%)** | *15.9-21.1* | 21.7 | 22.9 | 21.2 | 19.7 | 20.2 | 21.80 | 21.3 | 23.00 | 22.80 |
| **RET# (K/uL)** | *294-444* | 323 | 382.6 | 637.1 | **14.7** | **16.2** | **64.10** | **27.8** | 877.20 | 696.10 |
| **RET (%)** | *2.56-4.56* | 3.65 | 3.92 | 7.46 | 0.20 | 0.20 | 0.73 | 0.32 | 10.26 | 8.69 |
| **PLT (K/uL)** | *565-2159* | 858 | 657 | 645 | 1134 | 858 | 1209.00 | 661 | 866.00 | 839.00 |
| **PDW (fL)** | -- | 6.7 | 6.9 | 6.9 | 6.5 | 6.1 | 10.10 | 8.1 | 7.40 | 7.10 |
| **MPV (fL)**  | *4.3-6.1* | 6.0 | 6.0 | 6.2 | 5.9 | 5.5 | 7.70 | 7.2 | 6.70 | 6.30 |
| **WBC# (K/uL)** | *3.90-13.96* | 2.95 | 8.56 | 5.78 | 5.86 | 5.52 | 10.39 | 6.24 | 4.99 | 4.46 |
| **NEUT# (K/uL)** | *0.42-3.09* | 1.14 | 1.36 | 0.47 | 0.82 | 0.66 | 6.59 | 0.94 | 1.24 | 0.59 |
| **LYMPH# (K/uL)** | *2.88-11.15* | 1.52 | 6.42 | 4.93 | 4.50 | 4.54 | 2.43 | 5.00 | 3.58 | 3.32 |
| **MONO# (K/uL)** | *0.15-0.94* | 0.29 | 0.69 | 0.31 | 0.52 | 0.31 | 1.37 | 0.28 | 0.14 | 0.41 |
| **EO# (K/uL)** | *0.01-0.50* | 0.00 | 0.09 | 0.07 | 0.02 | 0.01 | 0.00 | 0.01 | 0.02 | 0.13 |
| **BASO# (K/uL)** | *0.00-0.14* | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.01 | 0.01 | 0.01 |
| **NEUT (%)** | *7.36-28.59* | 38.70 | 15.80 | 8.10 | 14.00 | 12.00 | 63.40 | 15.00 | 24.90 | 13.30 |
| **LYMPH (%)** | *61.26-87.12* | 51.50 | 75.00 | 85.30 | 76.80 | 82.20 | 23.40 | 80.10 | 71.70 | 74.40 |
| **MONO (%)** | *2.18-11.02* | 9.80 | 8.10 | 5.40 | 8.90 | 5.60 | 13.20 | 4.50 | 2.80 | 9.20 |
| **EO (%)** | *0.13-4.51* | 0.00 | 1.10 | 1.20 | 0.30 | 0.20 | 0.00 | 0.20 | 0.40 | 2.90 |
| **BASO (%)** | *0.01-1.26* | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.20 | 0.20 | 0.20 |

**Table S9. List of serum chemistry parameters evaluated by toxicology**

|  |  |
| --- | --- |
| Blood Urea Nitrogen (BUN)  | Albumin (ALB) |
| Creatinine (CREA)  | Globulin (GLOB) |
| BUN/CREA Ratio  | Albumin/Globulin (A/G) ratio) |
| Alkaline Phosphatase (ALP)  | Phosphorus (P) |
| Alkaline Aminotransferase (ALT)  | Calcium (CA) |
| Aspartate Aminotransferase (AST)  | Glucose (GLU) |
| Gamma-Glutamyl Transferase (GGT)  | Cholesterol (CHOL) |
| Direct Bilirubin (DBIL)  | Triglycerides (TRIG) |
| Total Protein (TP)  | Creatine Kinase (CK) |
| Total Carbon Dioxide (TCO2)  | Chloride (Cl) |
| Sodium (Na)  | Potassium (K) |
| Na/K Ratio  | Anion gap |

**Table S10.** Complete metabolic profiles of female mice injected with saline vehicle (**Control group**, *n*=3), DOX alone (**Treatment group #1**, 7 mg/kg/dose, cumulative dose 21 mg/kg, *n*=3), or DOX-C’ dots (**Treatment group #2**, 7 mg/kg/dose, cumulative dose 21 mg/kg, *n*=3).

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Sample** | ***Reference*** | ***#1******Saline*** | ***#2******Saline*** | ***#3******Saline*** | ***#1******DOX*** | ***#2******DOX*** | ***#3******DOX*** | ***#1******DOX-C’ dots*** | ***#2******DOX-C’ dots*** | ***#3******DOX-C’ dots*** |
|  | **Gender** |  | M | M | F | F | F | M | F | F | F |
|  | **Sacrifice Day** |  | *9* | *9* | *9* | *10* | *10* | *15* | *15* | *15* | *12* |
| **Urinary function** | **BUN (mg/dL)** | *5.0-28* | 35.0 | 16.0 | 16.0 | 16.0 | 22.0 | 21.0 | 24 | 20 | 20 |
| **CREA (mg/dL)** | *0.2-0.5* | 0.18 | 0.16 | 0.22 | 0.22 | 0.2 | 0.16 | 0.21 | 0.23 | 0.14 |
| **BUN/CREA**  | *-* | 194.4 | 100 | 72.7 | 72.7 | 110 | 181.3 | 114.3 | 87.0 | 142.9 |
| **Hepatic function** | **ALP (U/L)** | *105-370* | 121 | 115 | 128 | 142 | 145 | 52.0 | 155 | 133 | 155 |
| **ALT (U/L)** | *27-195* | 44.0 | 90.0 | 56.0 | 58.0 | 35 | 41.0 | 24.0 | 26.0 | 248 |
| **AST (U/L)** | *54-77* | 212 | 217 | 119 | 194 | 139 | 85.0 | 54.0 | 54.0 | 679 |
| **GGT (U/L)** | *-* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **DBIL (mg/dL)** | *-* | 0 | 0.1 | 0 | 0 | 0 | 0 | 0 | 0 | 0.2 |
| **TP (g/dL)** | *4.8-7.2* | 5.6 | 5.3 | 5.7 | 5.6 | 5.2 | 5.5 | 6.3 | 5.8 | 5.3 |
| **ALB (g/dL)** | *2.4-4.3* | 3.2 | 2.9 | 3.3 | 3.4 | 3.1 | 2.7 | 3.7 | 3.5 | 3.0 |
| **GLOB (g/dL)** | *1.7-2.2* | 2.4 | 2.4 | 2.4 | 2.2 | 2.1 | 2.8 | 2.6 | 2.3 | 2.3 |
| **A/G ratio** | *-* | 1.3 | 1.2 | 1.4 | 1.5 | 1.5 | 1.0 | 1.4 | 1.5 | 1.3 |
| **Metabolic profile** | **P (mg/dL)** | *7.3-14.5* | 6.9 | 9.0 | 8.9 | 9.8 | 9.1 | 8.5 | 9.9 | 9.8 | - |
| **Ca (mg/dL)** | *9.5-12.5* | 9.5 | 10.3 | 10.1 | 10.8 | 9.8 | 10.9 | 11.0 | 10.9 | - |
| **GLU (mg/dL)** | *172-372* | 139 | 137 | 138 | 178 | 184 | 176 | 224 | 244 | - |
| **CHOL (mg/dL)** | *55-169* | 113 | 62.0 | 91.0 | 87.0 | 103 | 111 | 88.0 | 70.0 | 84.0 |
| **TRIG (mg/dL)** | *67-289* | 75.0 | 40.0 | 72.0 | 100 | 75 | 95.0 | 62.0 | 80.0 | 67.0 |
| **CK (U/L)** | *428-1609* | 249 | 134 | 144 | 647 | 360 | 86.0 | 41.0 | 18.0 | - |
| **TCO2 (mEq/L)** | *-* | 30.0 | 35.0 | 27.0 | 28.0 | 27 | 14.0 | 18.0 | 14.0 | - |
| **Na (mEq/L)** | *145-181* | 158 | 158 | 154 | 166 | 155 | 160 | 157 | 157 | - |
| **K (mEq/L)** | *7.3-11.1* | 10.0 | 9.6 | 8.0 | 10.2 | 8.6 | 10.6 | 12.1 | 12.3 | - |
| **Cl (mEq/L)** | *111-134* | 115 | 109 | 109 | 118 | 110 | 113 | 115 | 115 | - |
| **Na/K ratio** | *-* | 16.0 | 16.0 | 19.0 | 16.0 | 18 | 15.0 | 13.0 | 13.0 | - |
| **Anion Gap** | *-* | 23.0 | 24.0 | 26.0 | 30.0 | 27 | 44.0 | 36.0 | 40.0 | - |

**Table S11.** Histopathology of triply-dosed tumor mice after *i.v.* injection of vehicle **(Control group**, *n*=3**),** DOX (**Treatment group #1**, 7 mg/kg/dose, cumulative dose 21 mg/kg, *n*=3) or DOX-C’ dots(**Treatment group #2**, 7 mg/kg/dose, cumulative dose 21 mg/kg, *n*=3).

|  |  |  |
| --- | --- | --- |
| **Treatment** | **Gross****Examination** | **Histologic Finding(s)** |
|  |  | ***Heart*** | ***Lungs*** | ***Liver*** | ***Kidneys*** | ***Spleen*** | ***Bone Marrow*** | ***Brain*** |
| **#1 Saline** | * *Lungs: Mottled, dark red, pink*
* *Kidneys: Mottled, pale*
 | *N* | *Alveolar histoocytosis, MF, 2.* | *N* | *N* | ***N*** | *N* | * *Glioma, hemorrhage*
 |
| **#2 Saline** | * *No gross changes*
 | *N* | *N* | * *Hepatitis, neutrophilic, histiocytic, and lymphocytic, with apoptosis, random, MF, 2.*
* *Hepatic arteriole hyperplasia, MF, 2.*
* *Kupffer cell hemosiderosis, MF, 2.*
 | * *Tubular degeneration, and necrosis, F, UL, 1.*
 | *N* |  *N* | * *Glioma, necrosis, hemorrhage*
 |
| **#3 Saline** | * *Liver: mild lobular pattern diffusely.*
 |  *N* | *N* | * *Hepatitis, neutrophilic, histiocytic, lymphocytic, random, MF, 2.*
 | *N* | * *Red pulp,*

 *erythroid*  *hematopoietic* *hyperplasia.* | *N* | * *Glioma, hemorrhage*
 |
| **#1 DOX** | * *No gross changes*
 | *N* | *N* | * *Hepatitis, neutrophilic, histiocytic, and lymphocytic, with hepatocyte apoptosis, random, MF, 2.*
 | *N* | *N* | * *Decreased cellularity, inc M:E ratio*
 | * *Glioma, hemorrhage*
 |
| **#2 DOX** | * *No gross changes*
 | *IVS, myocardial histiocytic infiltrate, F, 3.* |  *N* | *N* | *N* | *N* | * *Decreased cellularity, inc M:E ratio*
 | * *Glioma, necrosis, hemorrhage*
 |
| **#3 DOX** | * *No gross changes*
 | *N* | *NA* | *NA* | *NA* |  *NA* |  *NA* | * *Glioma*
 |
| **#1 DOX-C’ dots** | * *No gross changes*
 |  *N* |  *NA* | *NA* | *NA* |  *NA* | * *Decreased cellularity, inc M:E ratio*
 | * *Glioma*
 |
| **#2 DOX-C’ dots** | * *No gross changes*

 | *N* |  *NA* | *NA* | *NA* |  *NA* |  *N* | * *Glioma, hemorrhage*
 |
| **#3 DOX-C’ dots** | * *No gross changes*
 | *N* |  *NA* | *NA* | *NA* |  *NA* |  *N* | * *Glioma, hemorrhage*
 |

***Legend:*** *N: Normal; F: Focal; MF: Multifocal; D: Diffuse; UL: Unilateral; BL: Bilateral; 1: Minimal; 2: Mild; 3: Moderate; 4: Marked; U: Unavailable; NA: Not applicable; LV: Left ventricle; RV: Right ventricle; LA: Left atrium; RA: Right atrium; IVS: Interventricular septum; M:E: Myeloid:erythroid*