**Supplementary Table 1**. Human EFT, RMS, and NB cell lines used for current study.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Cell lines | Origin | Established post-chemo | References  (PMID) | STR Validation |
| Ewing’s family of tumor (EFT) | | | |  |
| 6647 |  |  |  | Unique |
| A673 | Unknown | Unknown | 1136433 | Unique |
| CB-AGPN |  |  |  | Unique |
| TC-106 |  |  | 21594428 | Unique |
| TC-138 |  |  |  | Unique |
| TC-205 |  |  |  | Unique |
| CHLA-9 | Thoracic tumor | No | 15289350 | Unique |
| CHLA-10 | Thoracic tumor | Yes | 15289350 | Unique |
| CHLA-32 | Pelvic tumor | No | 15289350 | Unique |
| CHLA-57 | Unknown | Unknown |  | Unique |
| CHLA-99 | Tumor | Unknown |  | Unique |
| COG-E-352 | Peripheral blood post mortem | Yes | 15289350 | Match to original material |
| CHLA-218 | Tumor | Yes |  | Unique |
| CHLA-56 | Bone Marrow | No |  | Match to original material |
| SK-N-MC | Retro-orbital metastasis | Yes |  | Unique |
| TC-71 | Humerus | Yes | 15289350 | Unique |
| TC-32 | Ileum and adjacent soft tissue | No | 15289350 | Unique |
| TC-248 |  |  |  | Unique |
| CHLA-25 | Unknown | Yes | 15289350 | Unique |
| CHLA-258 | Lung metastasis | Yes | 15289350 | Match to original material |
| Rhabdomyosarcomas (RMS) | | | |  |
| BIRCH | Alveolar, unknown | unknown | 7882340, 21316601 | Unique |
| Rh30 | Alveolar, bone marrow metastasis | No | 3691179 | Unique |
| CB-NJR | Alveolar, unknown | unknown | 2302710 | Unique |
| Rh28 | Alveolar, axillary lymph node metastasis | No | 3607778 | Unique |
| Rh18 | Embryonal, unknown | No | 9732232 | Unique |
| JR | Alveolar, unknown |  | 6950170, 22142829 | Unique |
| COG-R-466 | Tumor (mediastinum) | Yes | unpublished\* | Match to original material |
| TTC-442 | Embryonal, primary site | Unknown | 16849537, 19887553, 10602515, 7882340, 21316601 | Unique |
| Rh41 | Alveolar, lung metastasis | Yes | 19235922, 21882254  10918230, 17066459 | Unique |
| SMS-CTR | Embryonal, pelvic mass | Yes | 23882450 | Match to original material |
| RD | Embryonal, recurrent pelvic tumor | Yes | 4241949, 21316601 | Unique |
| TTC-516 | Embryonal, primary site | unknown | 23882450 | Unique |

\*Recently established cell line verified to be rhabdomyosarcoma, STR: Short-tandem repeat

**Supplementary Table 1.** Human EFT, RMS, and NB cell lines used for current study**-(continued).**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Cell lines | Origin | Previous treatment | References  (PMID) | TH expression | STR Validation |
| Neuroblastoma (NB) | | | |  |  |
| CHLA-122 | Tumor | No | 11507071 |  | Unique |
| CHLA-119 | Tumor | Yes | 11507071 |  | Unique |
| CHLA-136 | Tumor | Yes | 11507071 |  | Unique |
| CHLA-144 | Bone marrow | No | unpublished\* | TH(-)\*\* | Unique |
| CHLA-171 | Tumor | Yes | 11507071 |  |  |
| CHLA-172 | Tumor | Yes | 11507071 |  | Match to original patient material |
| CHLA-321 | Bone marrow | Yes | Unpublished\* | TH (+) | Match to original patient material |
| CHLA-42 | Bone marrow | No | 9850071 |  | Match to original patient material |
| CHLA-79 | Tumor | Yes | 9850071 |  | Unique |
| COG-N-316 | Bone marrow | No | unpublished\* | TH (+) | Match to original patient material |
| COG-N-415 | Blood | Yes | unpublished\* | TH (+) | Match to original patient material |
| LA-N-5 | Bone marrow | No | ATCC |  | Unique |
| SK-N-FI | Bone marrow | Yes | ATCC |  | Unique |
| SK-N-RA | Tumor | Yes | ATCC |  |  |
| SMS-KCN | Tumor | No | 9850071 |  | Match to original patient material |
| SMS-LHN | Relapsed tumor | Yes | 9850071 |  | Unique |
| CHLA-20 | Primary tumor | Yes | 9850071 |  | Unique |
| CHLA-291 | Bone marrow | Yes | unpublished\* | TH (+) | Match to original patient material |
| SK-N-BE(1) | Bone marrow | No | 9850071 |  | Matches to SK-N-BE(2) |
| CHLA-90 | Bone marrow | Yes | 9850071 |  | Unique |

\*Recently established verified to be neuroblastoma. TH: tyrosine hydroxylase, \*\* being confirmed: *MYCN* amplified and TERT (+).

**Supplementary Table 2.** Median Survival of xenograft mice bearing human pediatric cancer treated with irinotecan or nal-IRI.

|  |  |  |  |
| --- | --- | --- | --- |
| ***In vivo* model (type)** | **Median Survival (days)** | | |
| **Control** | **irinotecan** | **nal-IRI** |
| CHLA-258 (EFT) | 31.5  (n=8) | 66  (n=9)  *P* = 0.0006† | UD  (n=10)  *P* < 0.0001†  *P* = 0.0005\* |
| COG-E-352 (EFT) | 27  (n=10) | 98  (n=10)  *P* = 0.0033† | UD  (n=10)  *P* < 0.0001†  *P* < 0.04\* |
| CB-NJRm (RMS) | 13  (n=10) | 22  (n=10)  *P* = 0.0049† | 38.5  (n=10)  *P* < 0.0001†  *P* = 0.0001\* |
| SMS-CTRm (RMS) | 23  (n=9) | 27  (n=9)  *P* = 0.324† | 36  (n=9)  *P* = 0.09†  *P* = 0.22\* |
| CHLA-136 (NB) | 16  (n=9) | 36  (n=5)  *P* = 0.0169† | 85  (n=10)  *P* < 0.0001†  *P* < 0.0001\* |
| CHLA-79m (NB) | 13  (n=10) | 19  (n=9)  *P* = 0.02† | 47  (n=7)  *P* < 0.0001†  *P* = 0.0004\* |
| CHLA-32 (EFT) | 23  (n=9) | 27  (n=9)  *P* = 0.32† | 36  (n=9)  *P* = 0.01†  *P* = 0.33\* |
| Rh30 (RMS) | 31  (n=5) | 38  (n=5)  *P* = 0.20† | 62  (n=5)  *P* = 0.10†  *P* = 0.33\* |
| CHLA-119 (NB) | 20.5  (n=8) | 17.5  (n=4)  *P* = 0.93† | UD  (n=10)  *P* < 0.0001†  *P* < 0.0001\* |

UD: undefined, EFT: Ewing’s sarcoma family of tumors, RMS: rhabdomyosarcoma, NB: neuroblastoma, n: sample size, †: vs control, \*: vs irinotecan.

**Supplementary Table 3.** Pharmacokinetic variables for irinotecan and nal-IRI following intravenous injections in mice.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  |  | **Pharmacokinetic parameters** | | |  |
|  |  | **Dose** | **ke** | **T1/2** | **AUC0-inf** | **CLtot** | **Cmax** |
| **Formulation** | **Compound** | **(mg/kg)** | **(h-1)** | **(h)** | **(µg h/mL)** | **(mL/h)** | **(µg/mL)** |
| irinotecan | irinotecan | 5 | 0.7 | 1.1 | 0.3 | 299.9 | 0.3 |
|  |  | 10 | 0.7 | 0.9 | 1.5 | 138.8 | 1.6 |
|  |  | 20 | 0.8 | 0.8 | 3.0 | 137.6 | 2.8 |
|  | SN-38 | 5 | 0.7 | 1.1 | 0.3 | 370.0 | 0.6 |
|  |  | 10 | 0.7 | 1.0 | 0.7 | 308.7 | 1.4 |
|  |  | 20 | 0.6 | 1.1 | 0.8 | 510.6 | 1.4 |
| nal-IRI | irinotecan | 5 | 0.3 | 2.6 | 288 | 0.4 | 50.1 |
|  |  | 10 | 0.3 | 2.7 | 907 | 0.2 | 100.5 |
|  |  | 20 | 0.2 | 4.4 | 2609 | 0.2 | 197.0 |
|  | SN-38 | 5 | 0.2 | 3.4 | 43 | 2.4 | 3.7 |
|  |  | 10 | 0.2 | 3.7 | 121 | 1.7 | 7.2 |
|  |  | 20 | 0.1 | 5.7 | 345 | 1.2 | 21.1 |

ke, elimination rate constant; T1/2, elimination half-life; AUC 0-inf, area under the curve; CLtot, systemic clearance; Cmax, maximum drug concentration. Data are mean values.