Paediatric solid tumours arise from endodermal, ectodermal, or mesodermal lineages. Although the overall survival of children with solid tumours is 75%, that of children with recurrent disease is below 30%. To capture the complexity and diversity of paediatric solid tumours and establish new models of recurrent disease, here we develop a protocol to produce orthotopic patient-derived xenografts at diagnosis, recurrence, and autopsy. Tumour specimens were received from 168 patients, and 67 orthotopic patient-derived xenografts were established for 12 types of cancer. The origins of the patient-derived xenograft tumours were reflected in their gene-expression profiles and epigenomes. Genomic profiling of the tumours, including detailed clonal analysis, was performed to determine whether the clonal population in the xenograft recapitulated the patient’s tumour. We identified several drug vulnerabilities and showed that the combination of a WEE1 inhibitor (AZD1775), irinotecan, and vincristine can lead to complete response in multiple rhabdomyosarcoma orthotopic patient-derived xenograft tumours in vivo.

One barrier to identifying and validating biomarkers that predict sensitivity to molecularly targeted therapeutics is the lack of preclinical models that capture the diversity of paediatric solid tumours. For adult cancers, several important advances have been achieved in developing patient-derived organoids for colon, prostate, and pancreatic cancers, and this has led to an international collaboration called the Human Cancer Model Initiative for developing cancer and normal organoids for the research community. There are also international efforts to develop patient-derived xenografts (PDXs) for adult leukemias and solid tumours, including the EuroPDX consortium, the Public Repository of Xenografts, and the National Cancer Institute Patient-Derived Models Repository. Paediatric solid tumours are rare, relative to adult cancers, and access to tissue is a barrier to developing paediatric organoids or PDX models of solid tumours.

To obtain fresh tumour tissue from children with solid tumours, we developed a protocol (NCT01050296) called molecular analysis of solid tumours (MAST). Between 2010 and 2015, 225 patients consented to the protocol and we received 192 tumour specimens from 168 patients. Of the 192 specimens, 148 (77%) were injected into immunocompromised mice. In total, we injected 15 different types of paediatric solid tumour, including 41 neuroblastomas, 31 osteosarcomas, 20 rhabdomyosarcomas, 10 retinoblastomas, 9 Wilms tumours, 9 desmoplastic small round-cell tumours, 7 Ewing sarcomas, 6 high-grade sarcomas, and 5 adrenocortical carcinomas (Fig. 1b and Supplementary Table 1). Additionally, 10 tumour specimens representing 6 rare tumour types were injected. We have successfully established 67 O-PDXs from 12 different paediatric solid tumour types (Fig. 1b).

![Figure 1](https://example.com/figure1.png)
The overall engraftment efficiency was 45% (67/148) (Supplementary Table 1). The highest rates of engraftment were for high-grade sarcoma (83%), Wilms tumour (78%), retinoblastoma (70%), and rhabdomyosarcoma (65%) (Fig. 1b). Recurrent tumour samples were significantly more likely to engraft (63%) than were diagnostic samples (37%) (P = 0.012; Fig. 1c). There was a similar engraftment rate for samples from metastatic (53%) and primary (43%) sites (Fig. 1d). The engraftment efficiency of samples obtained before chemotherapy was similar (50%) to that of samples obtained during chemotherapy (41%) (Fig. 1e).

We performed haematoxylin and eosin (H&E) staining and immunohistochemistry on 51 O-PDX/patient tumour pairs (Extended Data Fig. 1a–d and Supplementary Table 2). Forty-nine of the tumours were evaluable for immunostaining, and 98% (48/49) were concordant between the O-PDX and the patient tumour. We scored the proportion evaluable for immunostaining, and 98% (48/49) were concordant (Fig. 1a–d and Supplementary Table 2). Forty-nine of the tumours were evaluated and photographed of the eight regions of the tumour in the O-PDXs showed that both were due to shifts in the clonal composition. The two mutations that were lost in the O-PDXs were detected in the O-PDX but not in the patient tumour and vice versa (Extended Data Fig. 2 and Supplementary Table 3). These differences in detection of mutations may have been due to patient tumour purity, clonal changes, or sequence coverage. To distinguish between these possibilities, we performed capture enrichment and Illumina sequencing for 32,113 single-nucleotide variations (SNVs) identified across the sample cohort. The two mutations that were lost in the O-PDXs showed that both were due to shifts in the clonal composition. Among the 14 mutations that were gained in the O-PDXs, 6 were not surveyed because of copy number alterations, 2 were due to low tumour purity, 2 were due to insufficient coverage in the patient tumours, and the O-PDXs, except for SJRHBO10928_X1, which was discordant by histopathological analysis.

Next, we surveyed 36 of the O-PDX tumours by transmission electron microscopy to characterize subcellular features of each tumour type (Supplementary Table 2). Rhabdomyosarcomas had intracellular myofilaments; neuroblastomas had dense core vesicles; liposarcomas had large lipid droplets; and osteosarcomas had swollen endoplasmic reticulum and collagen deposits (Extended Data Fig. 1f–i). Overall, the O-PDX tumours retained the cellular and subcellular features of the patient tumours (Supplementary Table 2).

To determine whether the O-PDX tumours retain the somatic mutations in the patient tumours and whether they acquire additional mutations, we performed whole-genome sequencing and whole-exome sequencing of the O-PDXs and the matched patient tumour and germline samples (51 O-PDX/tumour pairs). For some samples, mutations were detected in the O-PDX but not in the patient tumour and vice versa (Extended Data Fig. 2 and Supplementary Table 3). These differences in detection of mutations may have been due to patient tumour purity, clonal changes, or sequence coverage. To distinguish between these possibilities, we performed capture enrichment and Illumina sequencing for 32,113 single-nucleotide variations (SNVs) identified across the sample cohort. The two mutations that were lost in the O-PDXs showed that both were due to shifts in the clonal composition. Among the 14 mutations that were gained in the O-PDXs, 6 were not surveyed because of copy number alterations, 2 were due to low tumour purity, 1 was due to insufficient coverage in the patient tumours, and the O-PDXs, except for SJRHBO10928_X1, which was discordant by histopathological analysis.
of each state across all samples for that gene. For the bars that are half the height, the intensity is scaled, starting at 50% of maximum intensity.

tumour, and the remaining 5 were bona fide new SNVs acquired in the O-PDX. Together, these data suggest that the O-PDXs faithfully maintain many features of the genomic landscape of the patient tumour from which they were derived.

To determine whether the clonal composition of O-PDX tumours changed relative to the patient tumours, we performed clonal analysis using the deep-sequence reads for all the SNVs across 42 of the patient tumours and their matched O-PDXs (Supplementary Table 4).

In total, 24% (10/42) of the O-PDXs accurately preserved the clonal composition of the patient tumour (group 1; Fig. 2a). Thirty-one per cent (13/42) maintained the major clonal features of the patient tumour in the O-PDX but continued to clonally evolve in the mice (group 2; Fig. 2b). Some O-PDX tumours (33%; 14/42) were derived from the major clone in the patient tumour but showed evidence of clonal loss (group 3; Fig. 2c), and 12% (5/42) of the O-PDX tumours had clonal loss and were derived from a minor clone (<10% of patient tumour) (group 4; Fig. 2d). Osteosarcomas had the best clonal preservation and neuroblastomas had the least (Fig. 2e).

To determine whether the clonal composition of O-PDX tumours was stable with passages in vivo, we performed clonal analysis on 18 early-passage (initial engraftment or passage 1) and late-passage (passages 4–6) O-PDX tumour pairs (Supplementary Table 4). Forty-four per cent (8/18) of the O-PDX tumours had clonal preservation (group 1) at late-passage relative to early-passage (Fig. 2f), and a similar number (7/18) maintained the major clonal features of the early-passage tumour but continued to evolve (group 2).

We also analysed the different O-PDX sublines and regional clonal heterogeneity in the tumour mass. The patient tumours were initially implanted in up to ten immunocompromised mice, and each tumour that grew was designated as a separate subline. For this analysis, we selected 11 O-PDX tumours with multiple sublines (range two to five sublines per tumour) and performed clonal analysis (Supplementary Table 4). All tumours were from initial engraftment or the first passage. Sixty-four per cent (7/11) of the O-PDX models had clonal preservation across two sublines (group 1; Fig. 2g), and the remaining four had features of the major clone but showed some clonal evolution (group 2).

To test the regional heterogeneity within tumours, we selected six O-PDX tumours and sampled eight regions of the tumour using a biopsy punch (Fig. 2h, i). Clonal analysis of those samples showed clonal preservation (group 1; Fig. 2j and Supplementary Table 4).

To compare the gene-expression profiles of patient tumours with those of their matched O-PDXs, we performed RNA sequencing (RNA-seq) on all 102 samples that were analysed by whole-genome and whole-exome sequencing. We calculated the correlation coefficient from the log2-transformed values of fragments per kilobase of transcript per million mapped reads (FPKM) for each pair and grouped the data by tumour type (Extended Data Fig. 3a and Supplementary Table 5). There was no difference in the O-PDX/patient tumour correlation coefficients for the RNA-seq data by tumour type (P = 0.62, Kruskal–Wallis test). However, there was a significant inverse correlation between the patient tumour purity calculated from the whole-genome sequencing data and the O-PDX/patient tumour RNA-seq correlation coefficients for neuroblastoma and rhabdomyosarcoma (Extended Data Fig. 3b–d). Patient tumours with low tumour purity contained more infiltrating normal cells than those with higher tumour purity. Upon transplantation into immunocompromised mice, the normal cells were lost, which could lead to a lower correlation for the RNA-seq of the patient tumour and matched O-PDX sample (Extended Data Fig. 3).

To determine whether the developmental origins were preserved in the tumours, we identified the genes that were significantly upregulated in neuroblastoma, osteosarcoma, and rhabdomyosarcoma (Supplementary Table 5). The most significantly enriched pathways in neuroblastoma were those for neuronal development and homeostasis, rhabdomyosarcomas were enriched for myogenesis, and osteosarcomas were enriched for pathways in bone development and collagen production. To determine whether this association was reflected in the epigenetic landscape of the tumours, we performed chromatin immunoprecipitation followed by sequencing (ChIP–seq) for nine histone marks (H3K9/14Ac, H3K27Ac, H3K4me1, H3K4me2, H3K4me3, H3K9-14Ac, H3K9me2, H3K9me3, RNA Pol II, CTCF, and BRD4). We used chromatin prepared from a representative embryonal rhabdomyosarcoma (ERMS, SJRH000026_X1), an alveolar rhabdomyosarcoma (ARMS, SJRH010463_X16), an osteosarcoma (SJO5001112_X1), and a neuroblastoma.
We screened 16 rhabdomyosarcomas, 8 osteosarcomas, 4 neuroblastomas, 1 Ewing sarcoma, and 1 rhabdoid tumour. For comparison, we also included 21 paediatric solid tumour cell lines (Supplementary Table 6); 156 drugs were used in this analysis for a total of over 500,000 individual data points. Of the 1,960 plates screened, 49 (2.5%) had Z′ < 0 and were excluded from further analysis. Of the remaining plates, the average Z′ was 0.57, and 95% of all plates had Z′ between 0.27 and 0.82 (Extended Data Fig. 5a). Drugs with the same mechanism of action had similar activity across O-PDXs and cell lines (Extended Data Fig. 5b). Mutations in signal transduction pathways were predictive of tumour response in a subset of tumours (Extended Data Fig. 5c). Data were entered into a central database (https://stjude.org/cstn-drug-sensitivity), and curve fitting was performed to determine the half-maximum effective concentration (EC₅₀) of each drug for each tumour (Supplementary Table 6). Growth rate can influence drug sensitivity for some classes of drugs in high-throughput screening experiments, so we corrected for differences in growth rate (Supplementary Table 6). The most commonly used broad-spectrum chemotherapeutics were active across several tumour types, and histone deacetylase and proteasome inhibitors were particularly active across tumour types (Supplementary Table 6 and https://stjude.org/cstn-drug-sensitivity).

Among the drugs with tumour subtype-specific activity, the WEE1 inhibitor AZD1775 (MK-1775) was of particular interest for rhabdomyosarcoma because a phase I study of it with irinotecan (IRN) was recently completed by the Children’s Oncology Group (COG trial NCT02095132). This is important because IRN and vincristine (VCR) are used to treat recurrent rhabdomyosarcoma; and given the sensitivity of rhabdomyosarcoma O-PDXs to AZD1775, combining it with IRN and VCR may be justified for future clinical trials. Indeed, most of the rhabdomyosarcoma O-PDX tumours were sensitive to AZD1775, whether they were obtained at diagnosis or recurrence (Supplementary Table 6).

To establish a clinically relevant mouse-equivalent dose for bortezomib (proteasome inhibitor), panobinostat (histone deacetylase inhibitor), and AZD1775 (WEE1 inhibitor), we performed plasma and...
tumour pharmacokinetic studies of SJRH000026_X1 tumour-bearing mice (Extended Data Fig. 6a–c and Supplementary Information). Preclinical phase I studies were performed on athymic non-tumour-bearing mice to establish the tolerability of the drug combination at clinically relevant doses and schedules.14,15. We used O-PDXs derived from two high-risk ERMS tumours that were resected at recurrence (SJRHB000026_X1 and SJRH0B12_Y) and a high-risk ARMS tumour resected at recurrence (SJRHB013759_X1) (Supplementary Table 1). We introduced a luciferase reporter gene into the O-PDXs, orthotopically injected the tumour cells into immunocompromised mice and, several weeks later, enrolled the mice in a preclinical phase II trial as described previously.14,15. Mice were screened weekly by bioluminescence, and four to six courses (21 days per course) of therapy were administered (Extended Data Fig. 6d). Mice with a tumour burden greater than 20% of their body weight were removed from the study and scored as progressive disease. Stable disease, partial responses, and complete responses were scored as done previously.14,15. All of the mice treated with panobinostat and bortezomib had progressive disease for all three O-PDX tumours tested (Fig. 4a, b, Extended Data Fig. 6e and Supplementary Table 7). The mice treated with AZD1775 + VCR + IRN had a better response than those treated with VCR + IRN alone (Extended Data Fig. 6f–h and Supplementary Table 7).

On the basis of the activity of AZD1775 combined with VCR and IRN in multiple O-PDX rhabdomyosarcoma models, we performed a double-blind, randomized, placebo-controlled preclinical phase III study, as described previously.14,15. We used the same O-PDX models as above (SJRHB000026_X1, SJRH0B12_Y, SJRH0B13759_X1) and added another ARMS O-PDX (SJRHB013757_X2) (Supplementary Table 1). We enrolled 140 mice and randomized them to 4 treatment groups (placebo, AZD1775, VCR + IRN, and AZD1775 + VCR + IRN; Supplementary Table 8). Each of the O-PDX tumours had an improved outcome with the triple-drug combination relative to the standard of care (VCR + IRN), AZD1775 alone, or placebo (Fig. 4c–f). Individual O-PDX tumours showed marked differences in response to VCR + IRN and AZD1775 + VCR + IRN, highlighting the importance of using multiple O-PDX models for preclinical studies (Fig. 4e, f and Supplementary Table 8). In addition, we performed clonal analysis on 17 O-PDX tumours from SJRH000026_X1 mice with progressive disease in three different treatment groups (AZD1775, VCR + IRN, and AZD1775 + VCR + IRN). Fifty-nine per cent (10/17) of those tumours showed clonal selection for the minor clone (Supplementary Table 4), which was similar to results from previously published data on patient tumours before and after treatment.14,15.

Many of the O-PDXs that we have developed were from patients with recurrent disease, which is important because there were previously few models of recurrent paediatric solid tumours. The O-PDXs retained the molecular and cellular features of the patient tumour and the epigenetic landscape of their developmental origins. The clonal composition of the patient tumours was compared with the O-PDX tumours at early and late passages, from different sublines and distinct locations within the tumour mass. Some O-PDX tumours retained the clonal composition of the patient tumour even after several passages in mice but others underwent clonal changes. For some tumours, different sublines captured different clones from the patient tumour, suggesting that separately propagating individual sublines may be important for capturing the clonal heterogeneity in patient tumours. The drug screening identified chemotherapeutic agents that had broad activity as well as drugs that were more selective for particular cancer types. This approach led to the discovery that the combination of a WEE1 inhibitor with IRN and VCR can improve response across four different O-PDXs of high-risk rhabdomyosarcoma, including recurrent disease, relative to IRN and VCR alone. We have demonstrated clonal selection in the treated tumours, as shown previously in patients14,15 and it will be important to directly relate the clonal selection in O-PDX models to patients in future studies. This is proof of principle for using O-PDX tumours for basic and translational research on paediatric solid tumours and it provides a useful platform for understanding clonal selection in recurrent disease. All of our O-PDX models and associated data are freely shared through the Childhood Solid Tumour Network (www.stjude.org/CSTN/) with no obligation to collaborate.

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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METHODS

Power analysis was done to estimate the sample size for the preclinical phase III study. Cell lines were obtained from American Type Culture Collection (ATCC) and their identities were validated by analysing the short tandem repeats using the Promega PowerPlex 16 system. All cell lines were confirmed to be free of mycoplasma using the ATCC Universal Mycoplasma Detection kit. The preclinical phase II study was randomized but not blinded. The preclinical phase III study was randomized and blinded.

Animals. Athymic nude immunodeficient mice were purchased from Jackson Laboratories (strain code 007890). NSG mice were purchased from Jackson Laboratories (strain code 005557). C57BL/6 scid mice were purchased from Jackson Laboratories (strain code 001913). This study was performed in strict accordance with the recommendations in the Guide to Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Institutional Animal Care and Use Committee (IACUC) at St. Jude Children’s Research Hospital. All efforts were made to minimize suffering. All mice were housed in accordance with approved IACUC protocols. Animals were housed on a 12–12 light cycle (light on 6:00, off 18:00) and provided food and water ad libitum.

Patient consent and MAST protocol. Excess, de-identified tumour material was collected from patients with solid tumours at St. Jude Children’s Research Hospital in accordance with local institutional ethical regulations and institutional review board approval. Patient consent for tissue acquisition was obtained under the guidelines of the MAST protocol.

Primary patient tissue processing. Primary tumour tissue was processed for implantation within 2 h of surgical resection in most cases. Primary tumour was enzymatically dissociated into a single-cell suspension and injected into an anatomically correct location for the disease type when possible. If the initial tumour sample was too small for dissociation, tumour tissue was implanted in the flank location. Initial implantation was primarily done into recipient NSG female mice with the exception of retinoblastoma, which was implanted into C57BL/6 scid mice. After engrafment and sufficient tumour growth, the tumour was harvested and passedaged into athymic nude mice using the same dissociation and implantation techniques.

Orthotopic injections. Bone marrow injections. To minimize distress and movement during the procedure, isoflurane gas anaesthetic was used. The mouse was placed in the supine position on a nose-cone before injection. The skin of the knee joint was worked over with alternating iodine scrubs and 70% isopropyl alcohol wipes. The prep leg was flexed at the knee joint and secured to the work surface. The femur was palpated until the femoral condyles became visible. A 25-gauge needle on a 50 μl Hamilton glass syringe (Hamilton catalogue number 80920) was held at a 45° angle to the mouse, and the needle tip was inserted into the femur via the femoral intercondylar notch, while retracting the patella and patella tendon medially to avoid ligament damage. The needle was advanced down to the femoral head, which was approximately 5–10 mm depending on the size of the mouse, and 10 μl of cell suspension was slowly injected into the femur.

Intramuscular injections. The mouse was restrained gently but firmly by the scruff method. The rear foot nearest to the investigator was secured beneath the little finger and lower thumb. The area to be injected was swabbed with 70% ethanol. The needle was inserted, bevel up, into the caudal thigh at a 45° angle and 50–100 μl of cell suspension was slowly injected into the muscle while avoiding injury to the sciatic nerve.

Intravitreal injections. C57BL/6 scid mice were given general anesthesia via isoflurane inhalation continuously at 1–3% concentration with an oxygen flow rate of 2 l min⁻¹. The mouse was placed under the microscope where the eye was proptosed and a small incision using the tip of an 18-gauge needle was created and a small hollow 1.5 mm incision in the cornea was made using sterile small surgical scissors. The tip of the sterile scissors was inserted into the incision, directly over the flap, and the scissors were opened to introduce a pocket in the subcutaneous space. One individual piece of tumour tissue was inserted into the pocket using sterile forceps. One drop of 100 μl penicillin/streptomycin solution was inserted into the opening over the tissue piece. The incision was closed with Vetbond tissue adhesive (3M catalogue number 1469SB). The overlying skin was held together for 3–5 s with forceps to allow adequate time for drying.

Enzymatic tumour dissociation. Neuroblastoma. Tumour was minced with sterile scalpels and rinsed in phosphate buffered saline without calcium or magnesium (PBS-minus solution). Tumour suspension was transferred to a 50 ml conical tube and filled with PBS-minus solution. Dissociation was done by adding 600 μl of trypsin (10 mg ml⁻¹, Sigma catalogue number T9935) and the tube placed in 37 °C water bath for 10 min. Dissociation was stopped by adding 60 μl of soybean trypsin inhibitor (10 mg ml⁻¹, Sigma catalogue number T6522). DNase I (2 mg ml⁻¹, Sigma catalogue number D4513) and magnesium chloride (1 M) were added in equal amounts of 60 μl increments until tumour fragments easily settled at the bottom of the tube. Tumour suspension was filtered with a 40 μm cell strainer and then centrifuged at 500g (relative centrifugal force) for 5 min. Supernatant was discarded and 10 μl of red blood cell lysis solution (5 Prime catalogue number 2301310) added and allowed to incubate at room temperature for 10 min. A solution of PBS-minus (Lonza catalogue number 17-516F) 10% fetal bovine serum (FBS, Biowest catalogue number SO1520) was added to fill a 50 ml conical tube and the cell suspension centrifuged at 500 g for 5 min. Supernatant was discarded and the cell pellet was resuspended in PBS-minus/10% FBS for counting. Cells were then resuspended in Matrigel basement membrane matrix (BD Biosciences catalogue number 354234) at a concentration of 2 × 10⁶ cells per 10 μl and placed on ice for injection.

Soft tissue sarcoma. Tumour was placed through a tumour press and then rinsed with Dulbecco’s modified Eagle’s medium (DMEM) (Lonza catalogue number 12-604F). The tumour suspension was transferred to a 50 ml conical tube and filled with DMEM. Dissociation was done by adding 600 μl of trypsin (10 mg ml⁻¹; Sigma catalogue number T9935) and 50 μl of type II collagenase (275 U mg⁻¹; Worthington Biochemical catalogue number 4177), and then the tube was placed in a 37 °C water bath for 1 h. Dissociation was stopped by adding 600 μl of soybean trypsin inhibitor (10 mg ml⁻¹; Sigma catalogue number T6522). DNase I (2 mg ml⁻¹; Sigma catalogue number D4513) and magnesium chloride (1 M) were added in equal amounts of 60 μl increments until tumour fragments easily settled at the bottom of the tube. The tumour suspension was filtered with a 40 μm cell strainer and centrifuged at 500g for 5 min. The supernatant was discarded, 10 μl of red blood cell lysis solution (5 Prime catalogue number 2301310) was added and allowed to incubate at room temperature for 10 min. A solution of PBS-minus (Lonza catalogue number 17-516F)/10% FBS (Biowest catalogue number SO1520) was added to fill a 50 ml conical tube, and the cell suspension centrifuged at 500 g for 5 min. The supernatant was discarded and the cell pellet was resuspended in PBS-minus/10% FBS for counting. Cells were then resuspended in Matrigel (BD WorldWide catalogue number 354234) at a concentration of 1 × 10⁶ per 100 μl and placed on ice for injection.

Osteosarcoma. The tumour was placed through a tumour press and then rinsed with Dulbecco’s modified Eagle’s medium (17-516F). The tumour suspension was transferred to 100 ml screw cap glass bottle and filled with PBS-minus to the 100 ml mark. Dissociation was done by adding 600 μl of trypsin (10 mg ml⁻¹; Sigma catalogue number T9935) and 200 μg of type II collagenase (275 U mg⁻¹; Worthington Biochemical catalogue number 4177), and placed in a warm 37 °C water bath for 90 min, agitating with a magnetic bead at ~200 r.p.m. to have all tissue circulating and lift off the bottom of the glass bottle. Dissociation was stopped by adding 600 μl of soybean trypsin inhibitor (10 mg ml⁻¹; Sigma catalogue number T6522). DNase I (2 mg ml⁻¹; Sigma catalogue number D4513) and magnesium chloride (1 M) were added in equal amounts of 60 μl increments until tumour fragments easily settled at the bottom of the tube. The tumour suspension was filtered with a 40 μm cell strainer and centrifuged at 500g for 5 min. The supernatant was discarded, 10 μl of red blood cell lysis solution (5 Prime catalogue number 2301310) was added and allowed to incubate at room temperature for 10 min. A solution of PBS-minus (Lonza catalogue number 17-516F)/10% FBS (Biowest catalogue number SO1520) was added to fill a 50 ml conical tube, and the cell suspension centrifuged at 500g for 5 min. The supernatant was discarded, and the cell pellet was resuspended in PBS-minus/10% FBS for counting. Cells were then resuspended in Matrigel (BD WorldWide catalogue number 354234) at a concentration of 1 × 10⁶ per 100 μl and placed on ice for injection.
added to fill a 50 ml conical tube, and the cell suspension was centrifuged at 500 × g for 5 min. The supernatant was discarded, and the cell pellet was resuspended in PBS−/10% FBS for counting. Cells were then resuspended in Matrigel basement membrane matrix (BD Biosciences catalogue number 354234) at a concentration of 1 × 10^6 cells per 10 μl and placed on ice for injection.

Retinoblastoma. Tumour was minced with sterile scalpels and rinsed in RPMI (Lonza catalogue number 12-1676). Tumour suspension was transferred to a 50 ml conical tube and filled with RPMI. Dissociation was done by adding 600 μl of trypsin and placed on 37°C water bath for 10 min. Dissociation was stopped by adding 600 μl of soybean trypsin inhibitor (10 mg ml⁻¹, Sigma catalogue number T6222). DNaase I (2 mg ml⁻¹, Sigma catalogue number D3451) and magnesium chloride (1 M) were added in equal amounts of 60 μl increments until tumour fragments easily settled at the bottom of the tube. Tumour suspension was filtered with a 40 μm cell strainer and then centrifuged at 500 g for 5 min. Supernatant was discarded and 10 ml of red blood cell lysis solution (5 Prime catalogue number 2301310) added and allowed to incubate at room temperature for 10 min. A solution of PBS−/10% FBS (Bioxest catalogue number SO1520) was added to fill a 50 ml conical tube and the cell suspension centrifuged at 500 × g for 5 min. Supernatant was discarded and the cell pellet was resuspended in PBS−/10% FBS for counting. Cells were then resuspended in RPMI at a concentration of 1 × 10^5 cells per 5 μl for injection.

Tumour cryopreservation. After dissociation, tumour cells that were not used for passaging or high-throughput screening were cryopreserved for banking and later usage. Cells were counted and resuspended in chilled FBS/10% dimethylsulfoxide (DMSO) at a concentration of 6 × 10^6 cells per 1 ml per tube for all tumour types with the exception of retinoblastoma at a concentration of 1 × 10^6 cells per 1 ml. Cryo-tubes were placed in styrofoam containers and frozen at −80°C for 3 days and then transferred to liquid nitrogen for long-term storage.

Histopathological scoring. Histopathological features of the patient H&E-stained slides and the O-PDX-derived slides were compared for histological similarity. These patient-xenograft pairs were placed into similarity categories on the basis of evaluation of the following attributes: overall tumour cellularity, growth pattern, cytomorphology including degree of pleomorphism, and mitotic activity. The following classification system was used: same, no difference noted between patient and xenograft-derived samples; similar, changes in growth pattern and overall cellularity allowed with no difference in pleomorphism or mitotic activity; morphological shift, significant change such that the two lesions were morphologically dissimilar with significant difference in cellularity, cytomorphological features, degree of pleomorphism, growth pattern, and mitotic activity.

Immunohistochemical staining and scoring. Patient samples and the matched xenograft tissues were assessed for caspase 3 (total), caspase 3 (cleaved), and Ki-67. Whole-genome sequencing. Whole-genome sequencing and library construction were performed as described previously with the following modifications: 250–500 ng of genomic DNA was input for library construction using Illumina-compatible adapters, and four to six cycles of amplification were performed with KAPA HiFi Hotstart ReadyMix (KAPA Biosystems). Identification of single-nucleotide variants, copy number variants, structural variants, and indels was performed as described previously. Whole-exome sequencing. Whole-exome sequencing was conducted using the SeqCap EZ Hybrid Capture kit (Roche) in accordance with the manufacturer's instructions. RNA preparation and RNA-seq. RNA was isolated from individual TRiZol (Life Technologies) preparations via a phenol–chloroform extraction. Samples were first homogenized at 17,000 rpm for 30 s with a tissue homogenizer (Polytron, PT10-35GT). A 1:4 volume of chloroform (Sigma) was then added to each sample and incubated at room temperature for 3 min followed by centrifugation at 12,000 × g for 15 min. The aqueous layer was then transferred to a siliconized Eppendorf tube followed by the addition of 2 μl glycerol (Roche) and 500 μl isopropanol (Fisher). Samples were incubated at room temperature for 10 min followed by centrifugation at 12,000 × g for 15 min. Samples were then washed twice with ice-cold 80% ETOH (Fisher) to remove salts, resuspended in DEPC H₂O, and the concentration was determined with a Nanodrop (Thermoscientific). RNA was extracted from freshly isolated retinas using an RNasy Plus Mini kit (Qiagen catalogue number 74134). Libraries were prepared from approximately 500 ng total RNA with a TrueSeq Stranded Total RNA Library Prep Kit according to the manufacturer's directions (Illumina). Paired-end 100-cycle sequencing was performed on HiSeq 2000 or HiSeq 2500 sequencers, according to the manufacturer’s directions (Illumina). Gene-based FPKM quantification in RNA-seq was performed as described previously. Spearman correlations were calculated for each primary tumour-O-PDX pair. Clonal analysis. Targeted enrichment was performed using a Seqcap EZChoice Kit (Roche) according to the vendor’s instructions for the KAPA workflow with 500 ng of genomic DNA as the starting input for library construction. Clonal analysis was performed as described previously. To test for clonal changes within different regions of a single tumour, a subset of orthotopic xenograft tumours were harvested for segmental clonal analysis. The excised tumour was cut in half with the cut edge facing upwards. Each tumour half was visually segmented into four quadrants and a biopsy punch needle was used to sample the tissue in each quadrant, producing eight samples from a single tumour. Clonal analysis was performed for each of the eight quadrant samples as described above. Samples were divided into four groups on the basis of the tumour clonal evolution between primary and O-PDX tumours: group 1 O-PDX tumours retained the clonal structure of the primary tumour; group 2 O-PDX tumours acquired significant amount of new mutations compared with the matching primary tumour; a minor clone in the primary tumour was lost in O-PDX tumours in group 3 samples; in group 4 samples, O-PDX tumours were derived from a minor clone in the primary tumour.

ChIP–seq. SRJHHB000026_X1, SRJHHB010463_X16, SJ50001112_X1, and SJNBL046_X were submitted to Active Motif for ChIP–seq for nine histone marks (H3K27Ac, H3K27me3, H3K36me3, H3K4me1, H3K4me2, H3K4me3, H3K9-14Ac, H3K9me2, H3K9me3). RNA Pol II, CTCE, and BRD4. We first used BWA (version 0.5.9-r26-dev, default parameter) to align the ChIP–seq reads to human genome hg19 (GRCh37-lite). Picard (version 1.65(1600)) was then used for marking duplicated reads. Non-duplicated reads were kept for analysis by samtools (parameter ‘-l q -f 1024’ version 0.11.8 (r982-295)). We followed the ENCODE quality control criteria and used SPP (version 1.11) to draw cross-correlation, and calculated relative strand correlation values in R (version 2.14.0) with the packages caTools (version 1.17) and bitops (version 1.0-6) and estimated the fragment size. We required >10 million unique mapped reads for point-source factors (H3K4me2/3, H3K9/14Ac, H3K27ac, CTCE, RNA Pol II, BRD4) and >20 million unique mapped reads for broad markers (H3K9me3, H3K27me3, H3K36me3). We required 10 million unique mapped reads for INPUTs and relative strand correlation values <1. We noticed H3K4me1 is point-source factor at some stages while it is a broad mark in others. Therefore, we treated H3K4me1 as a broad mark in quality control analyses. Then, upon manual inspection of the cross-correlation plot generated by SPP, the best fragment size estimated (the smallest fragment size estimated by SPP in all our cases) was used to extend each read and generate a bigwig file to view on IGV (version 2.3.40). All profiles were manually inspected for clear peaks and good signal-to-noise separation.

Whole-exome sequencing. Whole-exome sequencing was conducted using the SeqCap EZ Hybrid Capture kit (Roche) in accordance with the manufacturer’s instructions. Haplotyping and genotyping. Haplotype analysis was performed using SPLINK (version 4.2) with settings as described above, and ChromHMM (version 1.10, with ‘-colfields 0,1,2,5 –center’ for broad markers (H3K9me3, H3K27me3, H3K36me3). We required 10 million unique mapped reads for INPUTs and relative strand correlation values <1. We noticed H3K4me1 is point-source factor at some stages while it is a broad mark in others. Therefore, we treated H3K4me1 as a broad mark in quality control analyses.
intensity by the maximum total percentage of a state covering a gene and flanking region. To determine the best region representing a gene, we first filtered annotated isoforms by TSS within 2 kb of any H3K4me3/H3K4me2/H3K27Ac/H3K9-14Ac peaks at any development stage; then we selected the highest expressed isoform at any development stage estimated by cuffdiff or the longest isoform if no expression level estimated by cuffdiff. Lastly, we reduced the interval for an HMM state to half bar and the intensity to half the normalized amount if it did not rank in the top 2 HMM state for a gene. As HMM states could be assigned by multiple genes, the maximum value across genes that used for normalization.

Percentages of HMM states were calculated for individual genes across four tumours with ChIP-seq profiles. Tumour-type-specific upregulated genes were selected on the basis of the following criteria: differentially expressed among tumour types (false discovery rate < 0.05), highly expressed in the specific tumour type (FPKM > 8), and highly overexpressed compared with other tumours (at least fourfold higher). Tumour-type-specific repressed genes were selected as follows: differentially expressed among tumour types (false discovery rate < 0.05), highly expressed in at least one other tumour type (FPKM > 8), and highly repressed compared with other tumours (at least fourfold lower). Tumour-type-specific epigenetically regulated genes were selected as tumour-specific upregulated or repressed genes with at least one state with a fraction change ≥ 0.25 between the specific and other tumours.

**Statistical analysis.** The P values were adjusted for multiple comparisons using the Benjamini and Hochberg method. To compare engraftment efficiency, we used MedCalc software (https://www.medcalc.org/calc/comparison_of_proportions.php) to compare proportions using the \( \chi^2 \) test as recommended in refs 21, 22. To compare time to engraftment, we used the comparison of means on MedCalc. Pre-study consultation for the preclinical phase III study was done with the Department of Biostatistics at St. Jude Children's Research Hospital. The data include a total of 140 mice treated in 4 different treatment groups from 4 O-PDX models. The goal of the study was to assess and compare the tumour response and tumour progression-free survival among treatment groups. Mice were randomized into each treatment group with a randomization code provided by the Department of Biostatistics. The survival curves of time to tumour progression were generated by the Kaplan–Meier method. log-rank tests were used to compare survival curves in each sub-group. Tumour response was defined by bioluminescence at the end of therapy as described below. If a mouse was taken off study at any point after enrolment because its tumour size was greater than 20% of its weight, it was automatically assessed a response of progressive disease. Bioluminescence signals less than 10^8 photons s^-1 cm^-2 sr^-1 were classified as a complete response. Animals with a signal between 10^-10^ to 10^9 photons s^-1 cm^-2 sr^-1 were classified as having partial response. Stable disease was the same as enrolment signal, 10^-10^ to 10^8 photons s^-1 cm^-2 sr^-1, and progressive disease was greater than 10^8 photons s^-1 cm^-2 sr^-1.

The observed response data are recorded in Supplementary Tables 7 and 8. The results showed that the triple combination AZD1755 + IRN + VCR had the highest complete response rate 40% (4/10) for SIRJHBO13757, X2, 90% (9/10) for SIRJHBO13759, X1, 37.5% (3/8) for SIRJHBO00026, X1, 22% (2/9) for SIRJHBO12, Y, 65% (13/20) for both ARMS O-PDXs, and 29% (5/17) for both ERMS O-PDXs combined. Time to tumour progression was defined as the time interval between the date of enrolment and the date of the tumour size being >20% of mouse weight or Xenogen signal >10 at the end of study. The survival probabilities at days 25, 66, and 126 for treatment groups are available upon request. Again, the triple combination AZD1755 + IRN + VCR had the highest progression-free survival probabilities; at the end of study (day 126) these were 100%, 100%, 62.5%, 55.6%, and 59% for SIRJHBO13757, X2, SIRJHBO13759, X1, SIRJHBO00026, X1, SIRJHBO12, Y, alveolar combined, and embryonal combined, respectively.

A subset of the Kaplan–Meier survival curves is given in Fig. 4 and the others are available in log-rank test. Dose response curves were fitted using the trapezoid rule. In the event of a failure to fit a sigmoidal dose–response curve in 384-well plates by running the CellTiter-Glo assay using three independent plates with alternating maximum/minimum/middle control signals on at least two different days, and then examined Z′ and other relevant statistics.

The maximum signal corresponded to cells treated with DMSO only; the minimum signal had cells treated with about 30 μM staurosporine; and the middle signal used the cell-line-specific EC50 value for staurosporine determined during the positive control selection described above. In general, we observed no systematic location-dependent effect across wells, the three signal levels were well separated, and Z′ values were above 0.4.

Cell culture experiments in dose–response. Cell lines were prepared by standard cell culture techniques. Xenograft cells were obtained from freshly dissociated tumours. Cell lines and O-PDX cells were counted and plated in 384-well plates (Corning catalogue number 8804BC); plating densities and plating conditions are shown in Supplementary Table 6. Twenty-four hours after plating, each cell line or O-PDX sample was drugged with both a compound plate(s) and a positive control plate using a Biomek FX (Beckman Coulter) liquid handler equipped with a pin tool. Compounds were obtained as dry powder and dissolved in DMSO to a target concentration of either 10 mM or 2 mM (for compounds with reported high cellular potency or solubility limitations). The pin tool transferred about 65 nl of compound stock, resulting in approximately 370-fold compound dilution. At 72 h after drugging, cells were lysed with the addition of 25 μl per well CellTiter-Glo reagent as described above. The compound plate contained compounds dissolved in DMSO arrayed in a 1:3-fold dilution series in columns 1–20 with 32 compounds per plate. The positive control plate was empty from columns 1–20; in columns 21–24, it contained single-point and I.3 serial dilutions of the primary and secondary positive controls (staurosporine and bortezomib, respectively) dissolved in DMSO to give about 10 mM top stock concentration, and wells containing DMSO alone (negative control). There were three technical replicates per biological replicate. The purity of each compound used in these studies was verified to be >95% using ultraviolet total wavelength content (UVTWC) and evaporative light-scattering detection spectroscopy, and the concentration of each solution was quantified using nitrogen chemo-luminescence where possible.

**Cell screening analysis.** Raw data processing: log2(RLU dose–response) fits. Raw luminescence RLU values for each compound at each concentration were log2-transformed and normalized to obtain the percentage activity using the following equation: 100 × [(mean(negctrl)− compound)/(mean(negctrl)− mean(posctrl)]

They were then pooled from replicate experiments before fitting. Here, negctrl and posctrl refer to the negative and positive controls on each plate. Outliers for controls were detected using the robust statistic below the [first quartile + 1.5 × IQR] and were excluded from further analysis. Of the remaining plates, the average Z′ was 0.57, and 95% of all plates had Z′ between 0.27 and 0.82.

In the event of a failure to fit a sigmoidal dose–response curve in 384-well plates by running the CellTiter-Glo protocol described above. Positive controls were selected on the basis of the compound's ability to achieve complete cell killing (RLUs near background levels) with a well-defined, sigmoidal dose–response curve shape. We selected staurosporine (primary) and bortezomib (secondary) as the positive controls for all drug sensitivity experiments.

For each cell line, we obtained a dose–response curve. For each drug, we used a four parameter model, assessed the variability of the assay in 384-well plates by running the CellTiter-Glo assay using three independent plates with alternating maximum/minimum/middle control signals on at least two different days, and then examined Z′ and other relevant statistics. The maximum signal corresponded to cells treated with DMSO only; the minimum signal had cells treated with about 30 μM staurosporine; and the middle signal used the cell-line-specific EC50 value for staurosporine determined during the positive control selection described above. In general, we observed no systematic location-dependent effect across wells, the three signal levels were well separated, and Z′ values were above 0.4.

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the smooth spline option in R was used to fit a curve that could be used to determine AUC.

**Pharmacokinetics.** AZD1775. The total plasma and tumour homogenate pharmacokinetics of AZD1775 in female tumour-bearing athymic nude mice (Jax Laboratories, aged 8–16 weeks) was assessed after a single oral gavage dose of 120 mg per kg (body weight). AZD1775 (AbMole, M2143, purity > 98%, molecular mass 500.60) was suspended in 0.5% methylcellulose (type 400 cPs, Sigma) at a nominal concentration of 12 mg ml \(^{-1}\) for a 10 ml kg \(^{-1}\) gavage volume. Mice were euthanized using an IACUC-approved method at 10 min and 1, 3, 24, and 72 h after dose, with three mice per time point. Whole blood was collected with sodium heparin via cardiac puncture, immediately centrifuged to plasma, and stored on dry ice for the remainder of study. Mice were then perfused with PBS via the aorta, the RS orthotopic xenografts excised from the hind limb, rinsed with PBS, and placed on dry ice. At the end of the in vivo procedures, all samples were transferred from dry ice and placed at −80 °C until analysis. Total plasma and tumour homogenate AZD1775 concentrations were assessed using a sensitive and specific liquid chromatography, tandem mass spectrometry assay. First, tissue samples were macerated, diluted with 1 ml of ultrapure water, and homogenized with a bead-based technique on a FastPrep-24 system (MP Biomedicals, Santa Ana, California, USA). Steel-lyzing matrix beads (MP Biomedicals, Metal Bead Lysing Matrix, 3 mg mg \(^{-1}\) of tissue) were added to the microcentrifuge tubes containing samples. The samples were then subjected to three vibratory cycles of 60 cycles per second of 1 min each on a FastPrep-24 system. To prevent overheating due to friction, samples were placed on wet ice for 5 min between each cycle. The homogenates were then stored at −80 °C until analysis. AZD1775 (AbMole, M2143, purity > 98%) stock solutions were prepared in methanol and used to spike matrix calibrators and quality controls. Plasma and tumour homogenate samples, 25 μl each, were protein precipitated with 100 μl of 5 mg ml \(^{-1}\) 1-cyano-1,2,3-tyramine (ApexBio, A8802, Batch 1, purity > 99%) in methanol as the extraction solvent. A 2 μl aliquot of the extracted supernatant was injected onto a Shimadzu LC-20A DxR high-performance liquid chromatography system via a LEAP CTC PAL autosampler. The liquid chromatography separation was performed using a Phenomenex Synergi Hydro-RP 80Å LC column (4.0 μm, 30 mm × 2.0 mm) maintained at 60 °C with gradient elution at a flow rate of 0.35 ml min \(^{-1}\). The binary mobile phase consisted of 0.1% formic acid in ultrapure water in reservoir A and 0.1% formic acid in methanol in reservoir B. The initial mobile phase consisted of 5% B with a linear increase to 70% B in 1.5 min. The column was then rinsed for 2.5 min at 100% B and then equilibrated at the initial conditions for 2 min for a total run time of 6 min. Under these conditions, the analyte and internal standard eluted at 0.98 and 2.56 min, respectively. Chromatographic peaks were identified by monitoring at 220 nm with the calibration standards run alongside. The chromatograms were processed using Shimadzu LC Solutions software and were weighted at 10% to reduce curve fitting artefacts. Two useful metrics were calculated from the dose–response curves on the basis of observed growth rates. ED\(_0\) was the effective dose required to achieve zero growth, or cytostatic behaviour. For curves that never reached zero observed growth rate, this value was set to the highest concentration tested. For curves from tumour models with a negative growth rate, this value was set to the lowest concentration tested. The second metric, AUC\(_{0\rightarrow t}\), was calculated as the area under the curve where the observed growth rate–dose–response curve was below zero.

**References.**
tandem mass spectrometry using a SCIEX API 4000 in positive ESI mode with monitoring of the following mass transitions: bortezomib 367.4 → 226.2, bortezomib-d8 374.8 → 234.0. The experimental bioanalytical runs were all found to be acceptable for a singlelicate non-good laboratory practice, preclinical pharmacokinetic assessment. A linear model (1/\(x^2\) weighting) fitted the calibrators across the 1–100 ng ml\(^{-1}\) range, with \(R \geq 0.99\). Above the calibration range, quality control samples were diluted with adequate precision and accuracy. The LLOQ was 1 ng ml\(^{-1}\) for plasma and 6 ng ml\(^{-1}\) for tissues because of the dilution factor. The intra-run precision and accuracy were <8.7–107%, respectively, across the matrices. The resultant bortezomib \(C_t\) data were grouped by matrix and time point, and manual imputation of data below the lower limit of quantitation (BLOQ) was as follows: if at any time point at least two-thirds of the \(C_t\) results were above the BLOQ, the BLOQ data were replaced with a value of \(1/2\) LLOQ, else the entire time point's data were treated as missing. Then, using Phoenix WinNonlin 6.4 (Certara USA, Princeton, New Jersey, USA), \(C_t\) data summary statistics (arithmetic mean, standard deviation, %CV, minimum, median, maximum) were generated, and the bortezomib arithmetic mean \(C_t\) data for each matrix were subjected to NCA. The extravascular model (Model 202) was applied, and AUC values were estimated using the ‘linear up log down’ trapezoidal rule. The terminal phase was defined as the two to three time points at the end of the \(C_t\) profile, and \(K_e\) was estimated using an unweighted log-linear regression of the terminal phase. \(T_{1/2}\) was estimated as 0.693/\(K_e\), and \(AUC_{\text{int}}\) was estimated as the AUC to the last time point (\(AUC_{\text{last}}\)) + predicted \(C_{\text{last}}/K_e\). Other NCA parameters estimated included observed maximum concentration (\(C_{\text{max}}\)), time of \(C_{\text{max}}\) (\(T_{\text{max}}\), concentration at the last observed time point (\(C_{\text{last}}\)), time of \(C_{\text{last}}\) (\(T_{\text{last}}\), apparent clearance (\(\text{CL} / F = \text{dose} / AUC_{\text{int}}\)), and apparent terminal volume of distribution (\(V_{\text{t}}/F\)). The average concentration over a dosing interval (\(C_{\text{avg}}\)) was estimated as \(AUC_{\text{dosing interval}} / \text{interval in hours}\). The apparent partition coefficient of bortezomib from the plasma to the tissue of interest (\(K_{\text{p.tissue}}\)) was estimated as the ratio of the \(AUC_{\text{tissue}}\) to \(AUC_{\text{int, plasma}}\) when available. To estimate a clinically relevant mouse dosage, the resultant mouse plasma \(AUC_{\text{int}}\) and \(C_{\text{avg}}\) were compared with the reported human plasma pharmacokinetic values at the putative single-agent bortezomib maximum tolerated dose at 1.3 mg m\(^{-2}\) intravenously on day 11 of therapy\(^2\). All inferences were made under the assumption of time-independent, linear, and dose-proportional pharmacokinetics in mice and humans.

Panobinostat. The total plasma and tissue pharmacokinetics of panobinostat in female tumour-bearing athymic nude mice (Jax Laboratories, aged 8–16 weeks) was assessed after a single intraperitoneal injection of 20 mg/kg body weight. Panobinostat (LC Labs, lot PNB-101, purity >99%, molecular mass 349.43) was suspended as the free base form in dextrose 5% for injection, United States Pharmacopeia (DSW, Baxter) at a nominal concentration of 2 mg ml\(^{-1}\), for a 10 ml per kg (body weight) injection volume. The intraperitoneal route was chosen given the low oral bioavailability reported in rodents\(^8\), and as it appeared to be the preferred route in reviewed mouse studies\(^9,8\). Mice were euthanized using an IACUC-approved method at 15 min, 40 min, and 2,25, 8, and 24 hr after dose, with three mice per time point. Whole blood was collected with sodium heparin by cardiac puncture, immediately centrifuged to plasma, and stored on dry ice for the remainder of the study. Mice were then perfused with PBS via the aorta, the RMs was orthotopic xenografts and tissues excised, rinsed with PBS, and placed on dry ice. At the end of the \textit{in vivo} procedures, all samples were transferred from dry ice and placed at –80 °C until analysis. Total plasma and tissue homogenate panobinostat concentrations were assessed using a sensitive and specific liquid chromatography, tandem mass spectrometry assay. First, tissue samples were macerated, diluted with a 5:1 volume (for tumour and brain) or a 3.1–7:0.1 (for large organs) volume of methanol and used to spike matrix calibrators and quality controls. Plasma and tissue homogenate samples, 25 μl each, were protein precipitated with 100 μl of 240 ng ml\(^{-1}\) panobinostat-d8 hydrochloride salt (Toronto Research Chemicals, P180502, lot 5-KSS-175-5, purity 96%) in methanol as an internal standard. A 2 μl aliquot of the extracted supernatant was injected onto a Shimadzu LC-20ADXR high-performance liquid chromatography system (Shimadzu, Kyoto, Japan) with a Waters XBridge BEH C18 LC column (2.5 μm, 50 mm x 2.1 mm) maintained at 60 °C with gradient elution at a flow rate of 0.35 ml min\(^{-1}\). The binary mobile phase consisted of ultrapure water–100 mM ammonium formate, pH 3.0–methanol (850:50:100 v/v/v) in reservoir A, and methanol–acetonitrile–100 mM ammonium formate, pH 3.0 (475:475:50 v/v/v) in reservoir B. The initial mobile phase consisted of 42.5% B and was maintained for 1.6 min. The column was then rinsed for 1.4 min at 100% B and then equilibrated at the initial conditions for 2 min for a total run time of 5 min. Under these conditions, the analyte and internal standard eluted at 0.85 and 0.83 min, respectively.

Analyte and internal standard were detected with tandem mass spectrometry using a SCIEX API 5500 Q-TRAP in positive ESI mode with monitoring of the following mass transitions: panobinostat 350.18 → 158.18, panobinostat-d8 357.22 → 156.18, and IRN, AZD1775 + VCR + IRN, and panobinostat + bortezomib. Mice were given four cycles of chemotherapy with 21 days per cycle. The chemotherapeutic drug combinations and schedules used were designed to mimic potential human clinical trials. Each treatment group contained three mice. Vincristine was dosed by intraperitoneal injection once on days 1, 5, and 8. Doxorubicin was dosed by intraperitoneal injection once daily on days 1–5 and 8–12. AZD1775 was dosed by oral gavage twice daily on days 1–5. Panobinostat was dosed by intraperitoneal injection once daily on days 1, 3, 5, 8, 10, and 12. Bortezomib was dosed by intraperitoneal injection once daily on days 1, 4, 8, and 11. Mouse weight and standard complete blood counts were monitored for each treatment group. The health of the animals was monitored daily throughout therapy.

Preclinical phase I. RMS orthotopic xenografts were created by injecting luciferase-labelled cells from SJRH800026_X1 (ERMS), SJRH8012_Y (ERMS), and SJRHB013759_X1 (ARMS) into recipient CD-1 nude mice using the intraperitoneal injection once daily on days 1, 3, 5, 8, 10, and 12. Bortezomib was dosed by intraperitoneal injection once daily on days 1, 4, 8, and 12. AZD1775 was dosed by oral gavage twice daily on days 1–5. Panobinostat was dosed by intraperitoneal injection once daily on days 1, 3, 5, 8, 10, and 12. Bortezomib was dosed by intraperitoneal injection once daily on days 1, 4, 8, and 11. Mouse weight and standard complete blood counts were monitored for each treatment group. The health of the animals was monitored daily throughout therapy.
(3 weeks per course) and bioluminescence was monitored weekly and at the end of therapy. Disease response was classified according to bioluminescence signal. Mice with a signal of 10^9 photons s^{-1} cm^{-2} sr^{-1} or less (similar to background) were classified as having complete response, 10^3–10^9 photons s^{-1} cm^{-2} sr^{-1} as having partial response, 10^2–10^4 photons s^{-1} cm^{-2} sr^{-1} (similar to enrolment signal) as having stable disease, and greater than 10^8 photons s^{-1} cm^{-2} sr^{-1} as having progressive disease. Mice with tumour burden at any time greater than 20% of body weight were also classified as having progressive disease. Mice were monitored daily while receiving chemotherapy.

**SJRHB012_Y (ERMS).** The AZD1775 groups and panobinostat + bortezomib groups were tested in two separate phase II preclinical trials. In the first trial, mice were randomized to four treatment groups: AZD1775 + VCR + IRN (low-dose protracted schedule), AZD1775 + VCR + IRN (standard 5-day schedule), VCR + IRN (low-dose protracted schedule), and placebo. The second trial randomly enrolled mice into two treatment groups, panobinostat + bortezomib, and placebo. The dose of AZD1775 was 60 mg per kg (body weight) oral gavage twice daily for days 1–5. IRN was 1.25 mg per kg (body weight) intraperitoneally once daily on days 1–5 and 8–12. Another regimen of IRN was also used in these mice at 3.125 mg per kg (body weight) intraperitoneally once daily on days 1–5. These represent the two most commonly used schedules in children in the clinic. Vincristine was administered at 0.38 mg per kg (body weight) intraperitoneally once daily on days 1, 8 and 15. Panobinostat was administered at 3 mg per kg intraperitoneally on days 1, 3, 5, 8, 10 and 12. Bortezomib was administered at 1 mg per kg (body weight) intraperitoneally once daily on days 1, 4, 8, and 11. Mice received six courses of chemotherapy, and bioluminescence was monitored weekly in the first trial and with each course (every 3 weeks) in the second trial. Disease response was classified according to bioluminescence signal. Mice with a signal of 10^9 photons s^{-1} cm^{-2} or less (similar to background) were classified as having complete response, 10^3–10^9 photons s^{-1} cm^{-2} sr^{-1} as having partial response, 10^2–10^4 photons s^{-1} cm^{-2} sr^{-1} (similar to enrolment signal) as having stable disease, and greater than 10^8 photons s^{-1} cm^{-2} sr^{-1} as having progressive disease. Mice with tumour burden at any time greater than 20% of body weight were also classified as having progressive disease. Mice were monitored daily while receiving chemotherapy.

**SJRHB013759_X1 (ARMS).** The AZD1775 groups and panobinostat + bortezomib groups were tested in two separate phase II preclinical trials. In the first trial, mice were randomized to three treatment groups: AZD1775 + VCR + IRN (low-dose protracted schedule), VCR + IRN (low-dose protracted schedule), and placebo. The second trial randomly enrolled mice into two treatment groups, panobinostat + bortezomib, and placebo. The dose of AZD1775 was 60 mg per kg (body weight) oral gavage twice daily for days 1–5. IRN was 1.25 mg per kg (body weight) intraperitoneally once daily on days 1–5 and 8–12. Vincristine was administered at 0.38 mg per kg (body weight) intraperitoneally once daily on days 1, 4, 8 and 15. Panobinostat was administered at 3 mg per kg intraperitoneally on days 1, 3, 5, 8, 10 and 12. Bortezomib was administered at 1 mg per kg (body weight) intraperitoneally once daily on days 1, 4, 8, and 11. Mice received six courses of chemotherapy, and bioluminescence was monitored weekly in the first trial and with each course (every 3 weeks) in the second trial. Disease response was classified according to bioluminescence signal. Mice with a signal of 10^9 photons s^{-1} cm^{-2} or less (similar to background) were classified as having complete response, 10^3–10^9 photons s^{-1} cm^{-2} sr^{-1} as having partial response, 10^2–10^4 photons s^{-1} cm^{-2} sr^{-1} (similar to enrolment signal) as having stable disease, and greater than 10^8 photons s^{-1} cm^{-2} sr^{-1} as having progressive disease. Mice with tumour burden at any time greater than 20% of body weight were also classified as having progressive disease. Mice were monitored daily while receiving chemotherapy.

**Preclinical phase III.** We performed a randomized, double-blind placebo-controlled preclinical phase III trial using 140 mice from 4 different O-PDX lines. RMS orthotopic xenografts were created by injecting luciferase-labelled cells from SJRHBO00026_X1 (ERMS), SJRHBO12_Y (ERMS), SJRHBO13757_X2 (ARMS), and SJRHBO13759_X1 (ARMS) into recipient athymic nude mice using the intramuscular injection technique previously described. Mice were screened weekly by Xenogen and the bioluminescence was measured. Mice were enrolled in the study after achieving a target bioluminescence signal of 10^9–10^10 photons s^{-1} cm^{-2} sr^{-1} or greater for 2 weeks or a palpable tumour, and chemotherapy was started the following Monday. Individuals giving the chemotherapy as well as those doing the bioluminescence imaging were blinded to the drugs being administered as well as to the specific O-PDX tumour line. The following drugs and doses were used in the preclinical phase III trial based on AUC-guided dosing obtained from pharmacokinetic studies matched to human doses that are clinically relevant in patients. The dose of AZD1775 was 48 mg per kg (body weight) oral gavage twice daily for days 1–5. IRN was 1.25 mg per kg (body weight) intraperitoneally once daily on days 1–5 and 8–12. Vincristine was administered at 0.38 mg per kg (body weight) intraperitoneally once daily on days 1, 8 and 15. Mice received six courses of chemotherapy (3 weeks per course) and bioluminescence was monitored on week 3 of each course and at the end of therapy. Disease response was classified according to bioluminescence signal as above. AZD1775 was reconstituted in 0.5% methylcellulose, IRN and VCR in sterile normal saline.

**Xenogen imaging and quantification.** Mice were given intraperitoneal injections of firefly D-Lucifer (Caliper Life Sciences 3 mg per mouse). Bioluminescent images were taken 5 minutes later using an IVIS 200 imaging system. Anaesthesia was administered throughout image acquisition (isoflurane 1.5% in oxygen delivered at 2 l min^{-1}). Living Image 4.3 software (Caliper Life Sciences) was used to generate a standard region of interest encompassing the largest tumour at maximal bioluminescence signal. The identical region of interest was used to determine the average radiance (in photons s^{-1} cm^{-2} sr^{-1}) for all xenografts.

**Data availability.** All sequence data have been deposited in the European Bioinformatics Institute (EMBL-EBI) under accession number EGAS00001002528.

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Extended Data Figure 1 | O-PDX models retain the cellular features of the corresponding patient tumours. a–d, Micrographs of H&E-stained patient tumours and corresponding O-PDX tumours for a representative rhabdomyosarcoma (a), liposarcoma (b), osteosarcoma (c), and neuroblastoma (d). e, Immunohistochemical staining for synaptophysin (brown) for SJNBL013761_D and the matched O-PDX. f–i, Transmission electron micrographs of a representative rhabdomyosarcoma (f), neuroblastoma (g), liposarcoma (h), and osteosarcoma (i). Inset for each micrograph is a higher magnification view of a key cellular hallmark of each tumour. HA, hydroxyapatite. Scale bars, a–e 40 μm; f–i 2 μm.
Extended Data Figure 2 | O-PDX tumours retain the genomic features of the patient tumours. a–d, Heat maps of the 51 tumours that had sufficient material for whole-genome sequencing and whole-exome sequencing analyses. Individual O-PDX/patient tumour pairs are indicated by alternating grey and white columns. Heat maps are grouped on the basis of the disease: rhabdomyosarcoma (a), osteosarcoma (b), neuroblastoma (c), and rare tumours (d).
Extended Data Figure 3 | Molecular and cellular clonal heterogeneity.

a, Boxplot of the correlation coefficient ($R$) between each O-PDX and the corresponding patient tumour. The error bars represent 1.5× the interquartile range. b–d, Scatterplots for the gene-expression correlation coefficients for the patient tumour compared with the O-PDX relative to the patient tumour purity determined from whole-genome sequencing. The Pearson correlation between those two variables is indicated for each tumour type. The line of best fit for the data is shown (red). e, f, Diagram and scatterplot of the clonal changes in SJOS001132 between the patient tumour and the O-PDX. The proportion of proliferating cells as measured by Ki67 immunostaining and dying cells as measured by cleaved caspase 3 immunostaining. The patient tumour had a major clone (80% of the tumour) with one cluster of SNVs (C1) and a minor clone (20% of the tumour) with two clusters of SNVs (C1 and C2). In the O-PDX, the minor clone was lost and the tumour continued to evolve and acquired an additional cluster of SNVs (C3). The total number of SNVs analysed in this sample was 238. g, H&E staining of the patient tumour and the O-PDX showing an expansion of cells with more aggressive pleiomorphic cellular features. h, i, Diagram and scatterplot of the clonal changes in SJNBL124 between the patient tumour and the O-PDX. The proportion of proliferating cells as measured by Ki67 immunostaining and dying cells as measured by cleaved caspase 3 immunostaining. The patient tumour had a major clone (80% of the tumour) with one cluster of SNVs (C1) and a minor clone (20% of the tumour) with two clusters of SNVs (C1 and C2). In the O-PDX, the minor clone was lost and the tumour continued to evolve and acquired an additional cluster of SNVs (C3). The total number of SNVs analysed in this sample was 373. j, In the H&E-stained patient tumour, there was a major clone (90% of the tumour) with proliferating small round cells and a minor clone (10% of the tumour) that had features of differentiated neuroblastoma cells. The cells with features of differentiated cells were lost in the O-PDX.
Extended Data Figure 4 | Epigenetic landscape reflects cellular origins. a, Heat map of the HMM states used in this study. b, Stack histogram of the percentage of each of the 16 HMM states for the genes expressed specifically in rhabdomyosarcoma across the 3 tumour types (osteosarcoma, neuroblastoma, rhabdomyosarcoma). c, Representative HMM and expression of a gene (MYOG) that is specifically expressed in rhabdomyosarcoma. d, e, Corresponding ChIP–seq peaks for all 12 antibodies and the input sample for the MYOG promoter in the rhabdomyosarcoma and osteosarcoma O-PDXs. f, Stack histogram of the percentage of each of the 16 HMM states for the genes expressed specifically in neuroblastoma across the 3 tumour types (osteosarcoma, neuroblastoma, rhabdomyosarcoma). g, Representative HMM and expression of a gene (PHOX2B) that is specifically expressed in neuroblastoma. h, i, Corresponding ChIP–seq peaks for all 12 antibodies and the input sample for the PHOX2B promoter in the neuroblastoma and rhabdomyosarcoma O-PDXs.

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Extended Data Figure 5 | Drug screening quality control. a, Box plot for the Z’ score for each of the 1,911 plates screened in this study that passed our quality control metrics. Most cell models (O-PDXs and cell lines) had a Z’ score above 0.4 (red line). The average Z’ was 0.57 and 95% of plates had Z’ between 0.27 and 0.82. The error bars represent 1.5 × the interquartile range. b, Scatterplot of AUC for the dose–response curves for pairs of drugs with similar and dissimilar mechanisms. Larger AUC values represent greater drug potency (killing). The Pearson correlation is shown for each drug pair. c, Scatterplot of AUC for the dose–response curve for two MEK inhibitors (trametinib and selumetinib). The cell models highlighted in red have mutations in the RAS/NF1/MEK pathway.
Extended Data Figure 6 | Preclinical pharmacokinetics and in vivo preclinical phase II. a–c, Concentration–time curves for panobinostat, bortezomib, and AZD1775 in mice bearing O-PDX tumours. Each data point is the mean and standard deviation of triplicate animals. The pharmacokinetics for IRN and VCR had been determined previously. d, Outline of the schedule used for the combination of panobinostat with bortezomib, VCR + IRN as the standard of care and AZD1775 + VCR + IRN. The schedules were selected to match that used in patients and the dose was selected to provide the same plasma exposure based on the pharmacokinetics in a–c. e–g, Tumour response for individual SJRHB000026_X1 mice on the preclinical phase II study for placebo-treated mice and each of the three treatment groups. Each line indicates an individual mouse with tumour. h, Stack bar plots of the response percentages for three different RMS O-PDX models with standard of care for recurrent RMS (IRN + VCR) and the AZD1775 + VCR + IRN treatment regimen. The numbers of mice in each response category are indicated on the plot.