**Bioluminescence imaging enhances analysis of drug responses in a patient-derived xenograft model of pediatric ALL**

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**SUPPLEMENTARY MATERIAL**

**SUPPLEMENTARY FIGURE LEGENDS**

**Supplementary Figure S1. Current scoring method for preclinical testing of novel agents.** Diagrammatic representations of scoring criteria employed by the PPTP, which are modeled after the clinical setting. PDXs achieving progressive or stable disease categories in response to a particular drug are considered Non-Responders, while those achieving partial, complete or maintained complete responses are considered to be Responders.

**Supplementary Figure S2. Correlations between log10 fold change and LGD or TT1%, and %huCD45+ cells in PB with LGD.** The median log10 fold change value for each treatment cohort was plotted against the respective leukemia growth delay (LGD) value for ALL-11-GL (A) and ALL-57-GL (B). The log10 fold change value for each treatment group was also plotted against respective time to 1% huCD45+ cells in PB (TT1%) values for ALL-11-GL (C) and ALL-57-GL (D). The PB values for each treatment group were also plotted against each group’s respective LGD values (E). R, Pearson correlation coefficient; P-value, two-tailed P-value of the Pearson correlation.

**Supplementary Figure S3. Determination of tumor volume doubling time (TVDT) and log cell kill equivalent (LCKe) values.** Leukemia development in control mice, as measured by the median %huCD45+ cells in PB, was plotted against the day post-treatment initiation at which engraftment was measured for ALL-11-GL (A) and ALL-57-GL (B). The TVDT values were determined by interpolating from the line of best fit the day at which three doublings had occurred, and dividing this value by three. LCKe values were then determined for ALL-11-GL (C) and ALL-57-GL (D) using the LGD values (treatment – control; T-C) for each treatment group using the equation LCKe = T-C/(3.32 x TVDT). R, Pearson correlation coefficient; P-value, two-tailed P-value of the Pearson correlation.

**Supplementary Figure S4. Comparison of engraftment rates between transduced and non-transduced PDXs.** Transduced ALL PDX cells and their relative passage-matched control cells were inoculated into NSG mice and engraftment monitored by flow cytometry (%huCD45+ vs. %muCD45+ cells in mouse peripheral blood). This comparison was performed during the second round of *in vivo* expansion of the transduced cells, therefore the transduced population had been selected via cell sorting to >99% GFP/Luciferase+ prior to inoculation. ALL-11 and ALL-11-GL cells (A) were inoculated into 4 mice per group, while ALL-57 and ALL-57-GL (B) were inoculated into 2 mice per group. One mouse in the ALL-57 group succumbed to a mouse thymoma at Week 12, therefore engraftment data for this mouse was not available beyond Week 12.

**Supplementary Figure S5. Comparison of gene expression profiles between transduced and non-transduced PDXs.** Potential alterations in gene expression profiles between ALL-11 and ALL-11-GL cells were investigated using an Illumina microarray. Transformation and quantile normalization of data were performed using the ‘LogTransform’ and ‘NormalizeColumns’ modules in GenePattern (<https://pwbc.garvan.org.au>). Gene expression differences between the 2 groups were analyzed using the GenePattern module ‘Multiplot’. R, Pearson correlation coefficient; P-value, two-tailed P-value of the Pearson correlation.

**SUPPLEMENTARY FIGURES**

**Figure S1.**

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**Figure S2.**



**Figure S3.**



**Figure S4.**



**Figure S5.**

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**SUPPLEMENTARY TABLES**

**Supplementary Table S1. Efficacy of VXL and venetoclax against ALL-11 and ALL-57 as measured by PB monitoring.**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Xenograft | Treatment | Median EFS (days) | | LGD (days) | Log-rank test  p-value | ORM |
|  |  | Vehicle control | VXL |  |  |  |
| ALL-11 | 1x VXL | 18.7 | 93.7 | 75.0 | <0.01 | MCR |
| 0.5x VXL | 44.1 | 25.4 | <0.01 | CR |
| 0.25x VXL | 35.3 | 16.6 | <0.01 | SD |
| 0.125x VXL | 28.9 | 10.2 | <0.01 | PD2 |
| venetoclax | 33.2 | 14.5 | <0.01 | CR |
| ALL-57 | 1x VXL | 19.2 | 66.9 | 47.7 | <0.01 | MCR |
| 0.5x VXL | 49.7 | 30.5 | <0.01 | CR |
| 0.25x VXL | 38.4 | 19.2 | <0.01 | CR |
| 0.125x VXL | 28.0 | 8.8 | 0.018 | PD1 |
| venetoclax | 26.0 | 6.8 | <0.01 | PD2 |

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Xenograft | Anatomical site | VXL dose | Median day 0 BLI, p/s/cm2/sr | Median lowest BLI output, p/s/cm2/sr | Log10 fold change |
| ALL-11 | Whole animal | 1x | 9.21E+09 | 4.07E+06 | -3.35 |
| 0.5x | 8.87E+09 | 1.16E+09 | -0.88 |
| 0.25x | 7.45+E09 | 7.97E+09 | +0.03 |
| 0.125x | 8.41E+09 | 2.01E+10 | +0.38 |
| Bone marrow | 1x | 1.52E+09 | 6.22E+05 | -3.39 |
| 0.5x | 1.93E+09 | 2.62E+08 | -0.87 |
| 0.25x | 1.60E+09 | 1.75E+09 | +0.04 |
| 0.125x | 1.56E+09 | 2.95E+09 | +0.27 |
| Spine | 1x | 4.89E+09 | 1.04E+06 | -3.67 |
| 0.5x | 4.55E+09 | 5.89E+08 | -0.89 |
| 0.25x | 3.71E+09 | 3.62E+09 | -0.01 |
| 0.125x | 4.68E+09 | 6.92E+09 | +0.17 |
| Brain | 1x | 1.37E+09 | 4.46E+05 | -3.49 |
| 0.5x | 1.43E+09 | 1.55E+08 | -0.97 |
| 0.25x | 1.10E+09 | 1.04E+09 | -0.02 |
| 0.125x | 1.84E+09 | 2.56E+09 | +0.14 |
| ALL-57 | Whole animal | 1x | 3.07E+10 | 1.26E+08 | -2.39 |
| 0.5x | 3.60E+10 | 9.59E+08 | -1.57 |
| 0.25x | 2.60E+10 | 2.63E+09 | -0.99 |
| 0.125x | 3.84E+10 | 1.71E+10 | -0.35 |
| Bone marrow | 1x | 2.47E+09 | 6.24E+06 | -2.60 |
| 0.5x | 2.38E+09 | 4.86E+07 | -1.69 |
| 0.25x | 2.04E+09 | 1.12E+08 | -1.26 |
| 0.125x | 1.88E+09 | 3.16E+08 | -0.77 |
| Spine | 1x | 1.51E+10 | 2.17E+07 | -2.84 |
| 0.5x | 1.37E+10 | 1.68E+08 | -1.91 |
| 0.25x | 1.01E+10 | 5.02E+08 | -1.30 |
| 0.125x | 1.24E+10 | 1.58E+09 | -0.90 |
| Brain | 1x | 4.30E+09 | 1.08E+07 | -2.60 |
| 0.5x | 3.90E+09 | 5.65E+07 | -1.84 |
| 0.25x | 3.18E+09 | 1.49E+08 | -1.33 |
| 0.125x | 4.69E+09 | 3.39E+08 | -1.14 |

**Supplementary Table S2. Log10 fold change data in response to treatment as measured by BLI for the whole animal, as well as individual sites of leukemic engraftment.**

**Supplementary Table S3. Log cell kill equivalent (LCKe) values for all treatment groups.**

|  |  |  |
| --- | --- | --- |
| Xenograft | Treatment | Log cell kill equivalent |
|  |  |  |
| ALL-11 | 1x VXL | 8.8 |
| 0.5x VXL | 2.9 |
| 0.25x VXL | 1.9 |
| 0.125x VXL | 1.2 |
| venetoclax | 1.7 |
| ALL-57 | 1x VXL | 5.8 |
| 0.5x VXL | 3.7 |
| 0.25x VXL | 2.3 |
| 0.125x VXL | 1.1 |
| venetoclax | 0.8 |