Testing effect of M4K ACVR1 inhibitors on an ACVR1 mutant and wild-type cell lines

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Aims

To test the ability of the latest M4K lead compounds to reduce the viability of patient-derived DIPG cell lines grown in 2D and 3D culture systems, and with or without gamma irradiation (to mimic current standard of care radiotherapy).

Procedure

Set up

Greiner 655090 flat bottomed plates were coated with laminin:

- Add 45µL of laminin to each well
- Incubate at least 2h at 37oC
- Remove laminin, rinse once in PBS, store in PBS at 4oC until use

Dilute M4K compounds to 10mM in DMSO. Store in aliquots sufficient for 3 biological repeats (+1 for error).

Day 0

Include multiple vehicle controls on each plate and a cell free repeat (to remove background signal).

2D system:

1500 HSJD-DIPG-007 and 6000 HSJD-DIPG-011 cells were seeded per well of laminin coated 96 well plates (three technical repeats of each compound, two vehicle controls per plate). Each in 100µL TSM-C per well (see previous protocol for medium recipe). Include

3D system:

40 HSJD-DIPG-007 and 500 HSJD-DIPG-011 were seeded per well in Nexcelom ultra low attachment U-bottomed 384 well plates. Each in 40µL TSM-C per well. Four technical repeats of each compound were seeded.

Day 1

Dilute M4K compound to 2mM in TSM-B/C (50x dilution), then to 2µM in TSM-C (+ 1:500 propidium iodide to mark cell death). Add 100µL (96 well plate) or 40µL (384 well plate) of each compound dilution to each well (2x dilution to a final concentration of 1µM), include a vehicle control (same dilution with DMSO only making a final concentration of 0.01%).

Day 3

Irradiate cells with 4Gy gamma radiation.

Day 7

Scan cells with image cytometer to record final confluence/sphere diameter and propidium iodide intensity. Harvest cells with CellTiter-Glo® 3D (for all cells and all conditions) as a measure of culture viability according to manufacturer's instructions.

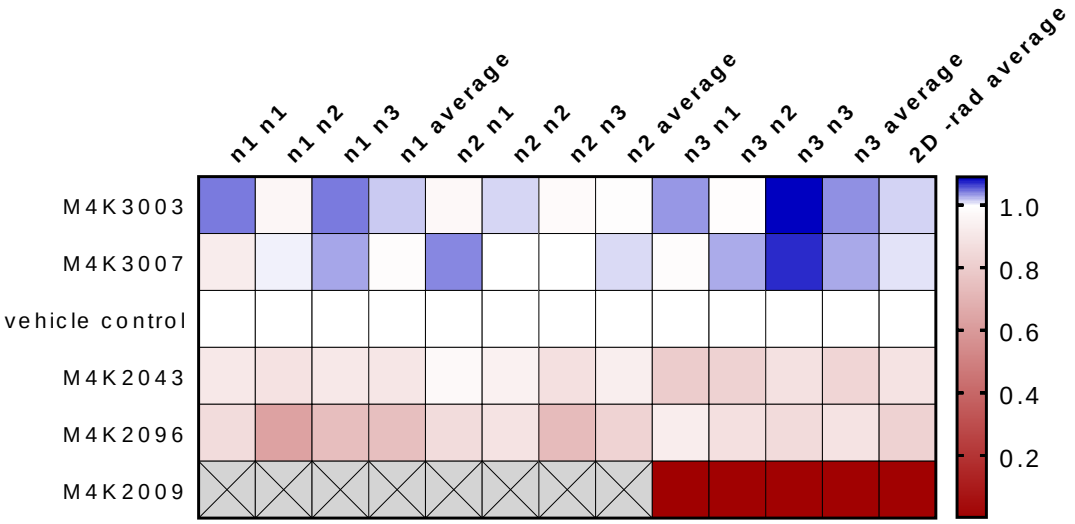
Data analysis

All values were normalised to the average of the vehicle controls on the plate (or the plate divided into sections using the vehicle controls, if visible variation across the plate).

Results

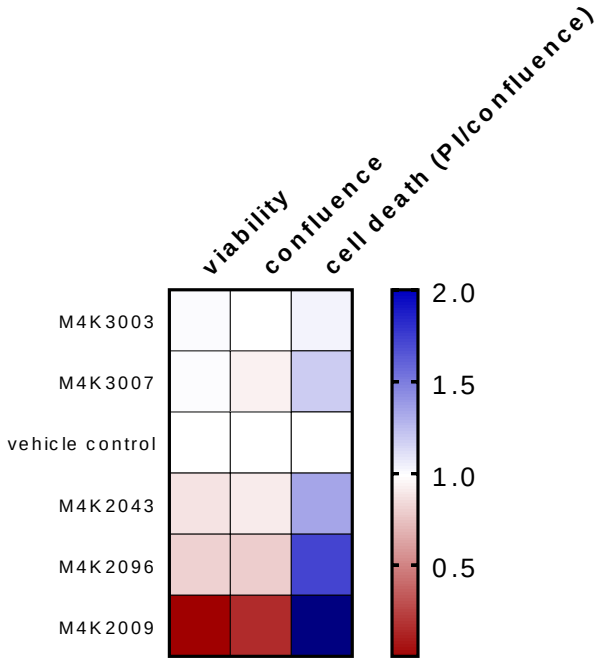
In the mutant ACVR1 cell line HSJD-DIPG-007 the most effective probe is M4K2009 (with the caveat that this dataset only includes one biological repeat), with all M4K2000 series compounds being more effective than M4K3000 series compounds (Figure 1). Comparison of additional end point read outs such as confluency or cell death supports this ranking (Figure 2). Consideration of the effectiveness of each compound in different growth conditions i.e. 2D vs 3D and with or without radiation treatment suggests that M4K2043 and M4K2096 may be less effective in addition to radiation, however M4K2009 remains highly efficient at killing HSJD-DIPG-007 cells (Figure 3). Results in the ACVR1 WT HSJD-DIPG-011 cell line were more variable but indicate that M4K2009 is less effective, with M4K2096 in this case being the most effective probe (Figure 4). These contrasting screen results could indicate that the effectiveness of M4K ACVR1 inhibitory compounds could be dependent on the mutations present. For the HSJD-DIPG-011 cell line, in two out of three biological repeat viability and confluence results are concurrent (Figure 5). Comparison of growth conditions in the HSJD-DIPG-011 cell line is difficult because of the variation between biological repeats, but the data is included in Figure 6.

Figure 1 – End point viability of cells following 7 day treatment with M4K compounds.



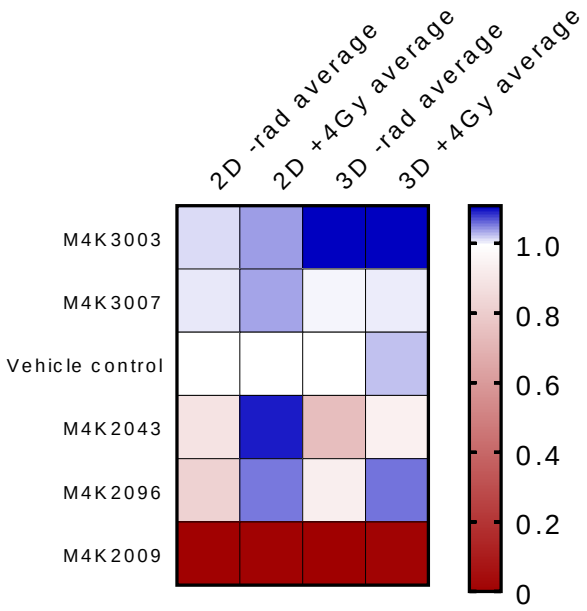
A heatmap showing the end point viability of HSJD-DIPG-007 cells (ACVR1^{R206H}, H3.3^{K27M}) grown for 7 days in 2D in the presence of 1μM of the indicated M4K compound. Viability was quantified with the CellTiter-Glo assay and normalised to the average of two vehicle controls. Columns are individual technical repeats (n[biological repeat] n[technical repeat]), averages of one biological repeat (n1-3 average) or the overall average (2D –rad average).

Figure 2 – Assay end point comparison for ACVR1 mutant DIPG cells



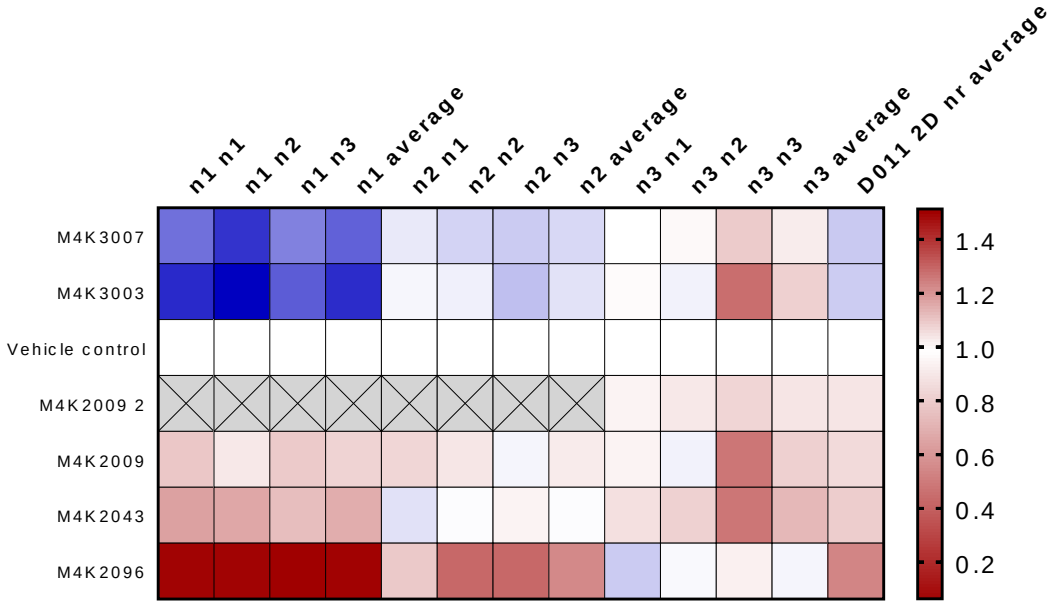
A heatmap showing the end point viability, confluence or cell death of HSJD-DIPG-007 cells (ACVR1^{R206H}, H3.3^{K27M}) grown for 7 days in 2D in the presence of 1µM of the indicated M4K compound. All values were normalised to the average of two vehicle controls. Values are averages of three biological repeat (Except M4K2009, which is only one biological repeat).

Figure 3 – Growth condition comparison for ACVR1 mutant DIPG cells



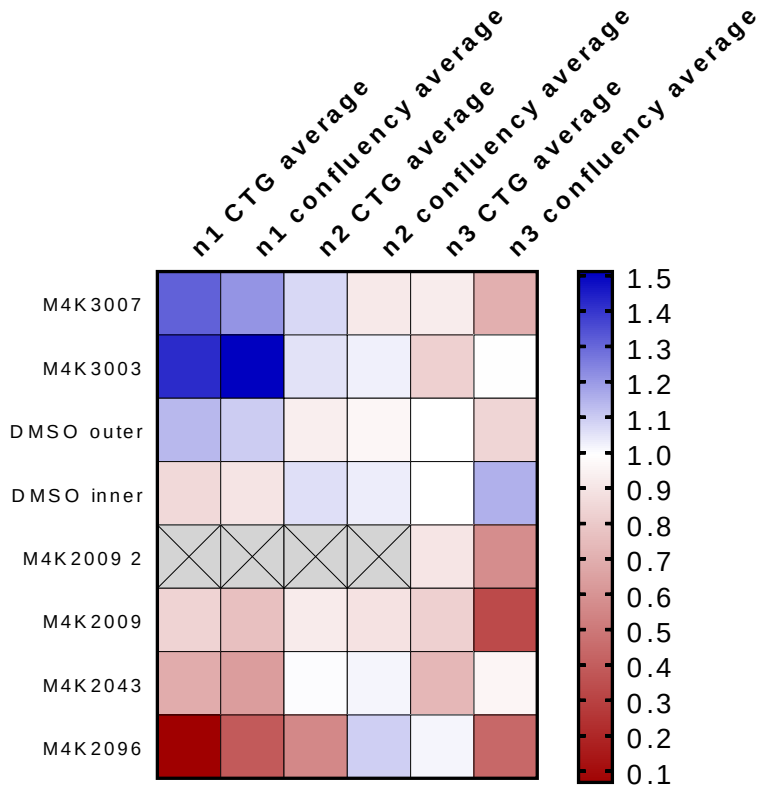
A heatmap showing the end point viability of HSJD-DIPG-007 cells (ACVR1^{R206H}, H3.3^{K27M}) grown for 7 days in the presence of 1µM of the indicated M4K compound. Cells were grown either in 2D or 3D and either without radiation treatment (-rad) or with 4Gy of gamma radiation (+4Gy). End point viability was quantified with the CellTiter-Glo assay and normalised to the average of two vehicle controls. Values are the average of three biological repeats (except M4K2009 for which only one biological repeat was completed).

Figure 4 – End point viability of ACVR1 WT cells with M4K compounds



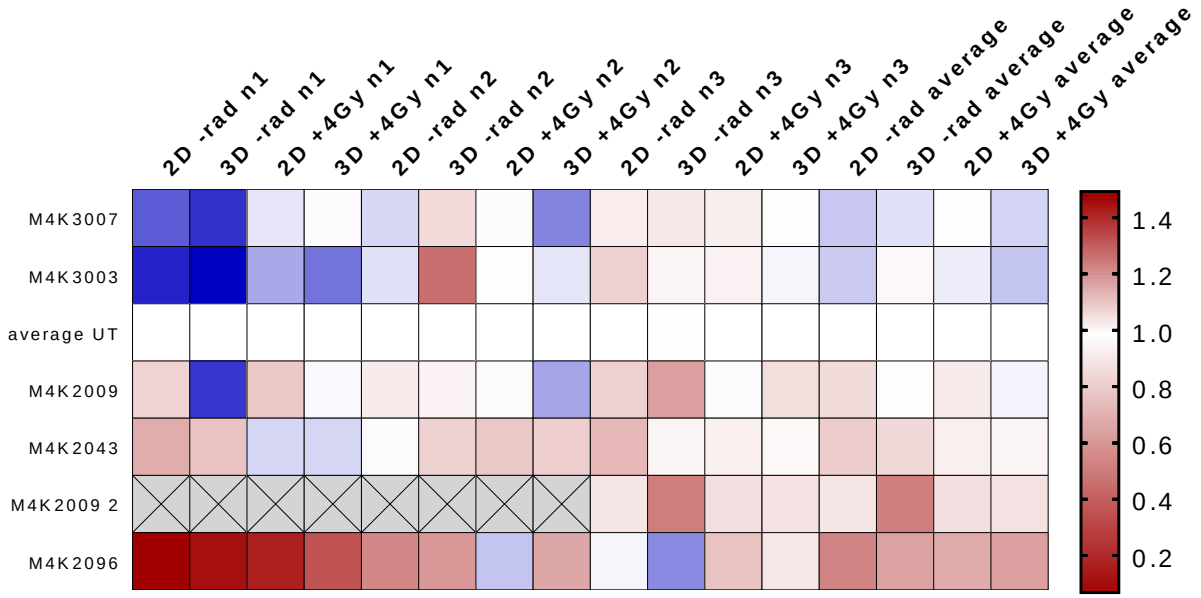
A heatmap showing the end point viability of HSJD-DIPG-011 cells (ACVR1^{WT}, H3.3^{K27M}) grown for 7 days in 2D in the presence of 1µM of the indicated M4K compound. Viability was quantified with the CellTiter-Glo assay and normalised to the average of two vehicle controls. Columns are individual technical repeats (n[biological repeat] n[technical repeat]), averages of one biological repeat (n1-3 average) or the overall average (2D –rad average).

Figure 5 – Assay end point comparison for ACVR1 WT cells



A heatmap showing the end point viability, confluence or cell death of HSJD-DIPG-011 cells (ACVR1^{WT}, H3.3^{K27M}) grown for 7 days in 2D in the presence of 1µM of the indicated M4K compound. All values were normalised to the average of two vehicle controls. Values are averages of three technical repeats of the inidcated biological repeat (n1-3).

Figure 6 – Growth condition comparison for ACVR1 WT cells



A heatmap showing the end point viability of HSJD-DIPG-011 cells (ACVR1^{WT}, H3.3^{K27M}) grown for 7 days in the presence of 1µM of the indicated M4K compound. Cells were grown either in 2D or 3D and either without radiation treatment (-rad) or with 4Gy of gamma radiation (+4Gy). End point viability was quantified with the CellTiter-Glo assay and normalised to the average of two vehicle controls. Values are individual technical repeats from the indicated biological repeat (n1-3).