Discovery of potent, selective, and orally available Wee1 inhibitors that demonstrate increased DNA damage, tumor cell mitosis and in vivo tumor regression

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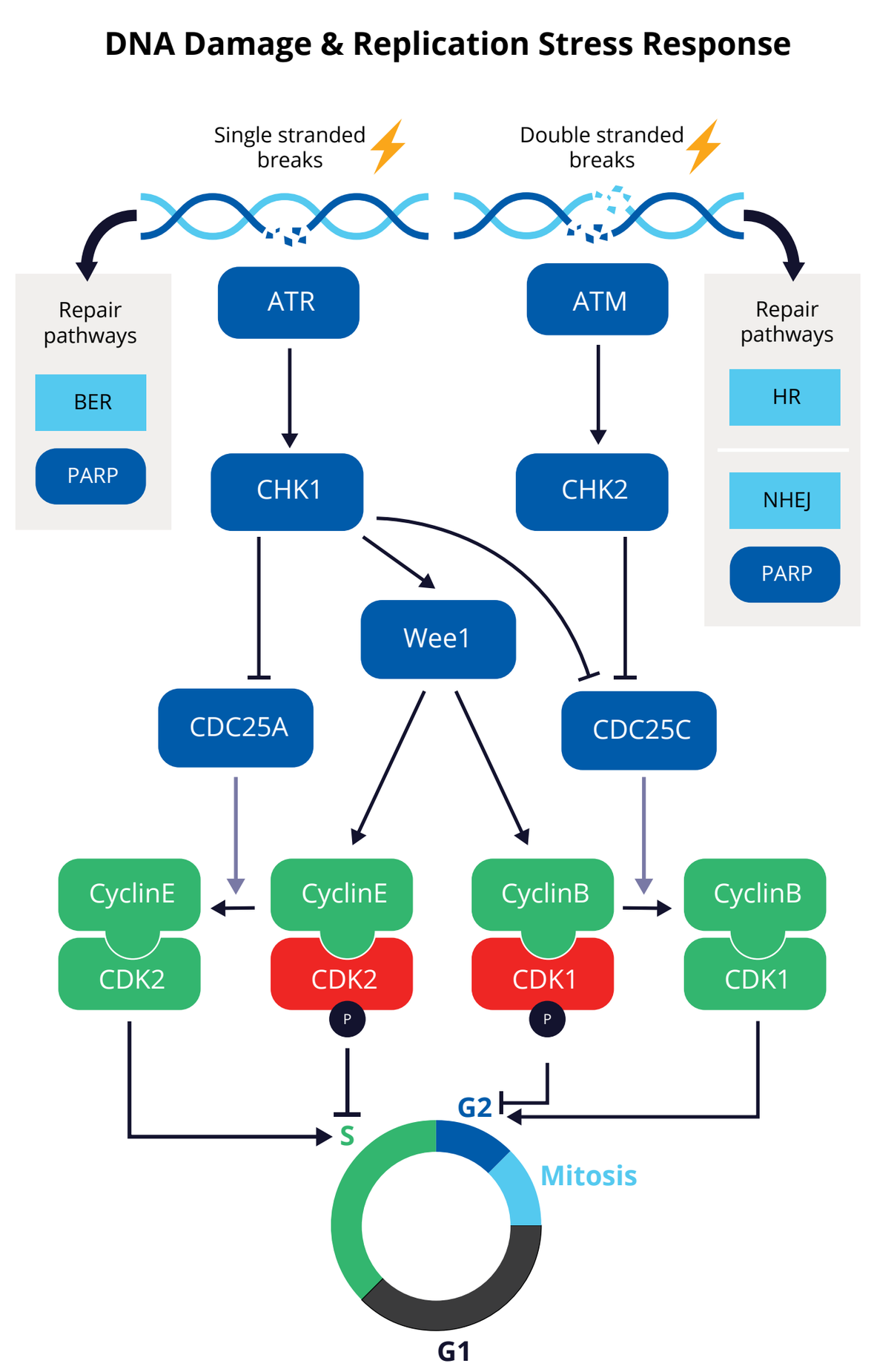
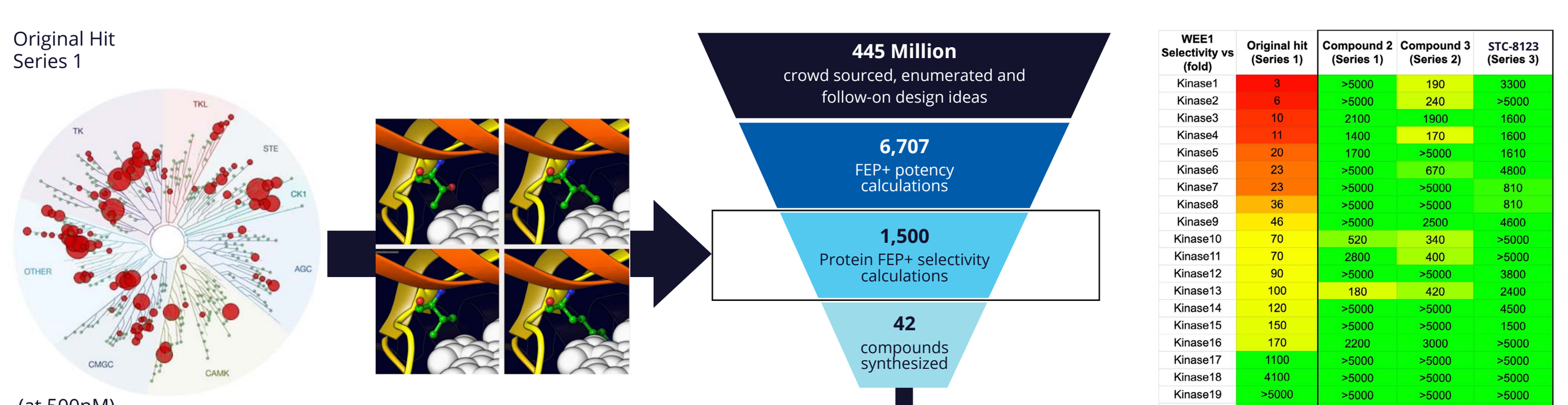
Introduction

- In the presence of errors or damage during DNA replication, cell cycle checkpoints (e.g. ATR, CHK1, and Wee1) and repair machinery (e.g. PARP) work in concert to delay cell cycle progression until sufficient repair has been achieved
- In cancer cells, elevated levels of replication stress caused by oncogene activation, loss of tumor suppressors, and defects in the DNA repair machinery lead to an increased reliance on cell cycle checkpoints such as Wee1
- In response to DNA damage and activated replication stress response, Wee1 inhibits the activation of both CDK2 (CDC1) and CDK1 (CDC2) through the phosphorylation of Tyrosine 15, allowing DNA damage repair prior to mitotic entry
- Wee1 inhibition releases cell cycle brakes, resulting in unsustainable levels of DNA damage, premature catastrophic mitosis, and tumor cell apoptosis

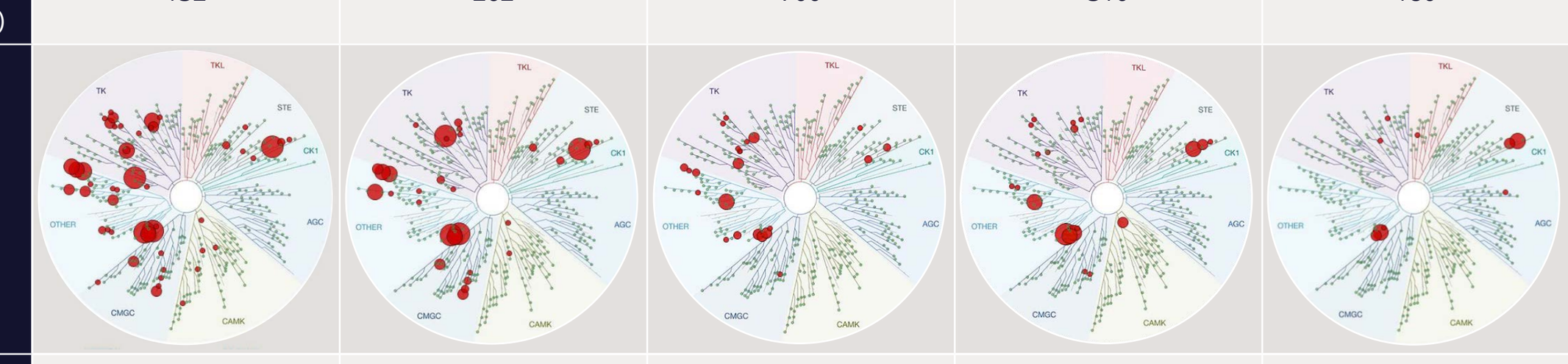


Rapid Optimization of Gene-Family Wide Selectivity: Schrödinger's FEP+ / Protein FEP+ Workflow Enabled One-Step Optimization of Non-selective Hit Into Multiple Selective Series



In-house 20 kinase panel curated based on protein FEP+ hypothesis supported rapid compound selectivity assessment and correlated well prospectively with kinase wide selectivity as assessed by ScanMAX.

Schrödinger Wee1 Inhibitors Have Similar Potency and Improved Kinase Selectivity Compared with Competitor Compounds

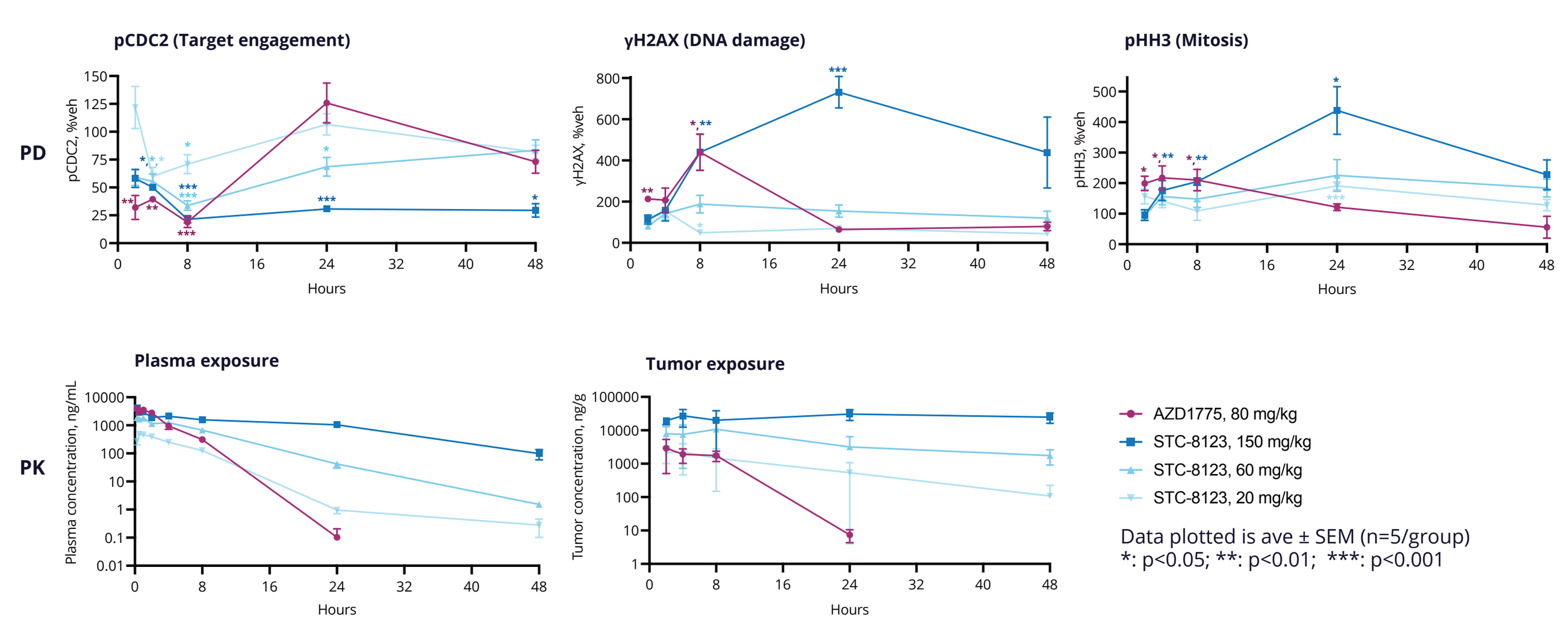
	AZD1775	Zn-C3	Compound 2 (Series 1)	Compound 3 (Series 2)	STC-8123 (Series 3)
Wee1 biochemical IC50 (nM)	0.4	0.7	0.9	0.8	1.2
Cellular target engagement (pCDC2 MSD) in A427 cells (nM)	132	262	700	310	180
Kinome Selectivity scanMAX					
scanMAX screening conc.	1μM	1μM	500nM	500nM	1μM

Multiple structurally distinct chemical series which are highly potent and selective for Wee1 were quickly discovered using Schrödinger's computational platform. STC-8123 was profiled at 1 μM across a panel of over 450 kinases. STC-8123 shows exquisite selectivity for Wee1 in this assay panel, binding significantly, with a greater than 90% inhibition relative to control, to only 8 other kinases.

Anti-Proliferative Activity in a Variety of Tumor Cell Lines Representing Several Tumor Types

	Cell viability CellTiterGlo (CTG) IC_{50} (nM)						
Tumor type	Lung	Ovarian		Breast		Colon	
Cell line	A427 (3 days)	OVCAR3 (3 days)	SKOV3 (7 days)	HCC1806 (5 days)	MDA-MB-436 (7 days)	SW620 (7 days)	SW1463 (10 days)
AZD1775	210	130	30	250	710	410	220
STC-8123	290	440	80	300	890	300	240

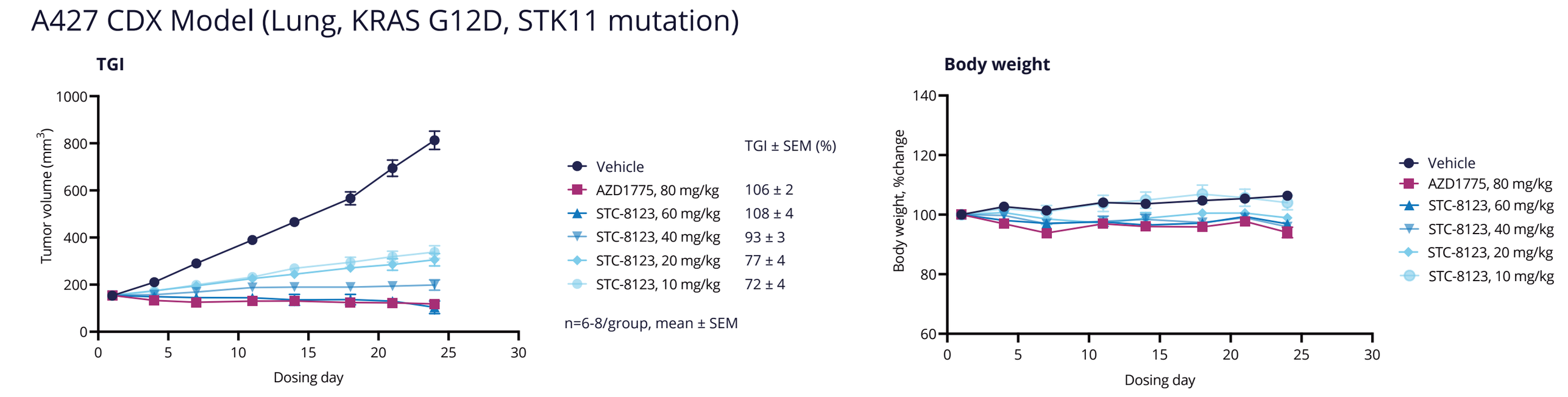
Robust PD Marker Modulation and PK/PD Relationship In Vivo in A427 Tumor Model

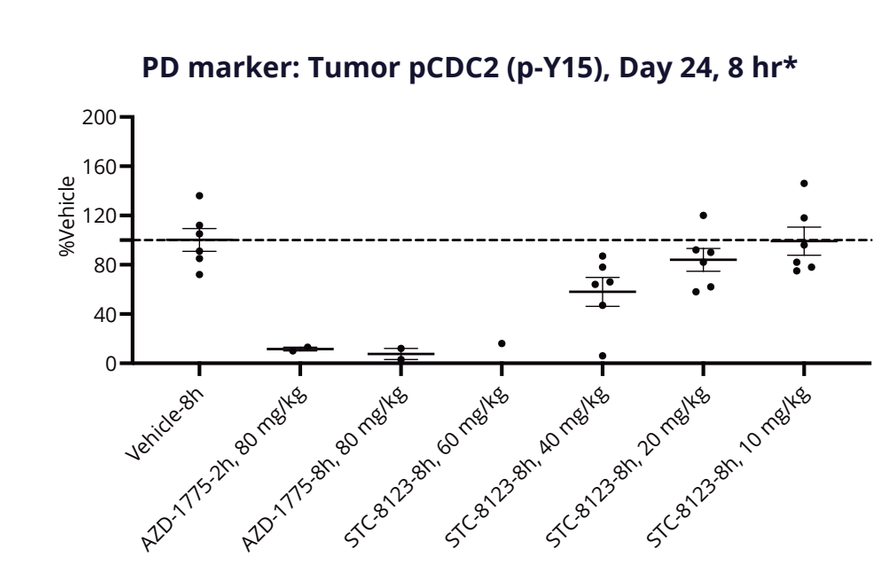


SCID NOD mice were inoculated with A427 non small cell lung cancer cells. After reaching 300-400 mm^3 in size, mice received a single dose of vehicle, 80 mg/kg AZD1775, or STC-8123 at three doses. Tumor samples were analyzed for downstream markers: pCDC2 by MSD, γH2AX by HTRF, and Phosphohistone H3 (pHH3) by Western Blot. Plasma and tumor exposure for AZD1775 were below the quantifiable limit at 48 hours.

STC-8123 is Well Tolerated and Demonstrates Robust Tumor Growth Inhibition (TGI) in Tumor-bearing Mouse Models

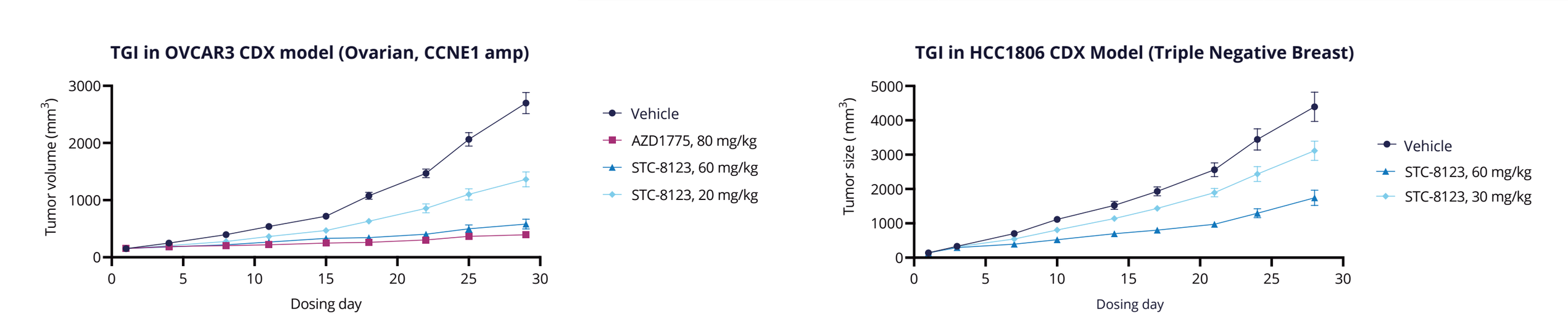
A427 CDX Model (Lung, KRAS G12D, STK11 mutation)





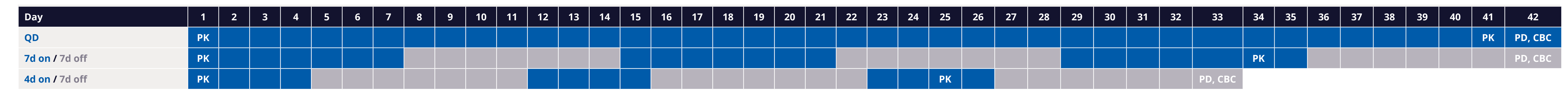
STC-8123 Tumor and Plasma Exposure

Group	Exposure	
	Plasma AUC_{last} (ug*h/mL), day 1 / day 23	**Tumor** (ng/g) ± SEM, day 24, 8 hr
AZD1775, 80 mg/kg	15.8 / 11.5	4.6 ± 1.1
STC-8123, 60 mg/kg	12.9 / 11.4	38.3 ± 4.0
STC-8123, 40 mg/kg	6.0 / 7.2	11.5 ± 2.6
STC-8123, 20 mg/kg	2.3 / 3.0	3.1 ± 1.2
STC-8123, 10 mg/kg	0.7 / 1.1	0.1 ± .03

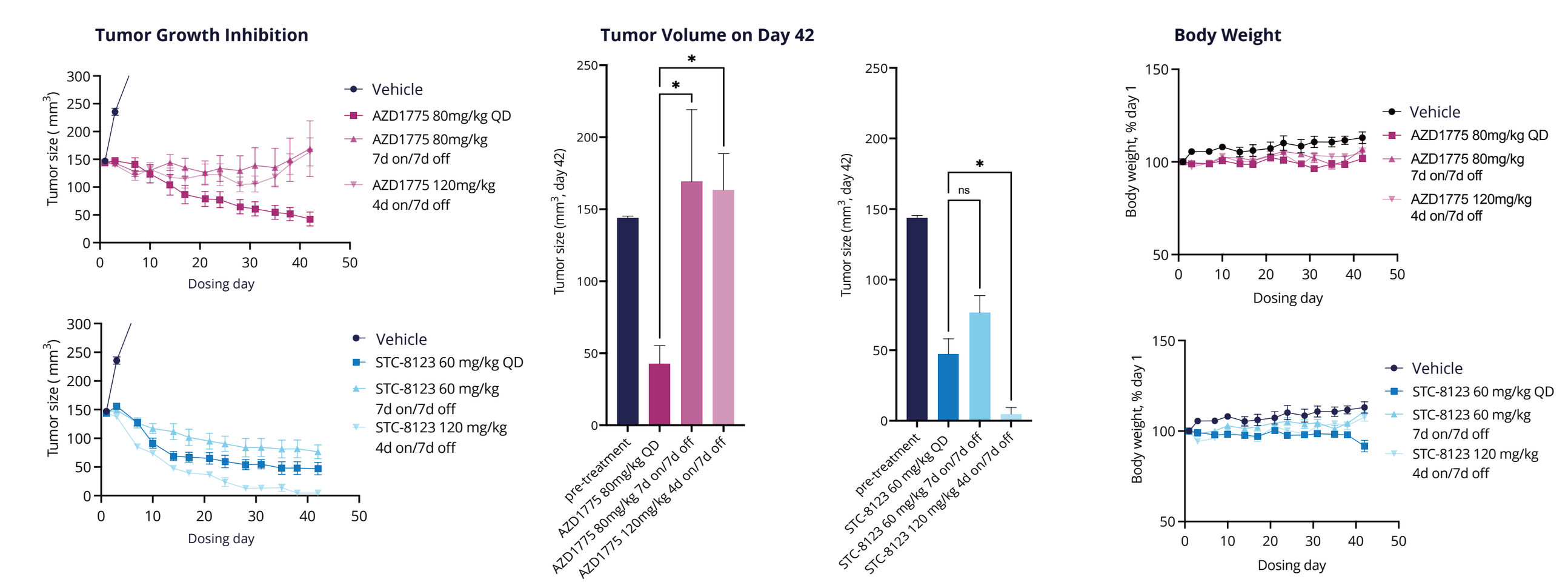


*Dose dependent PD effects observed in tumor samples. In highest dose condition, only one tumor sample available for analysis.

Dosing Holiday Study Design

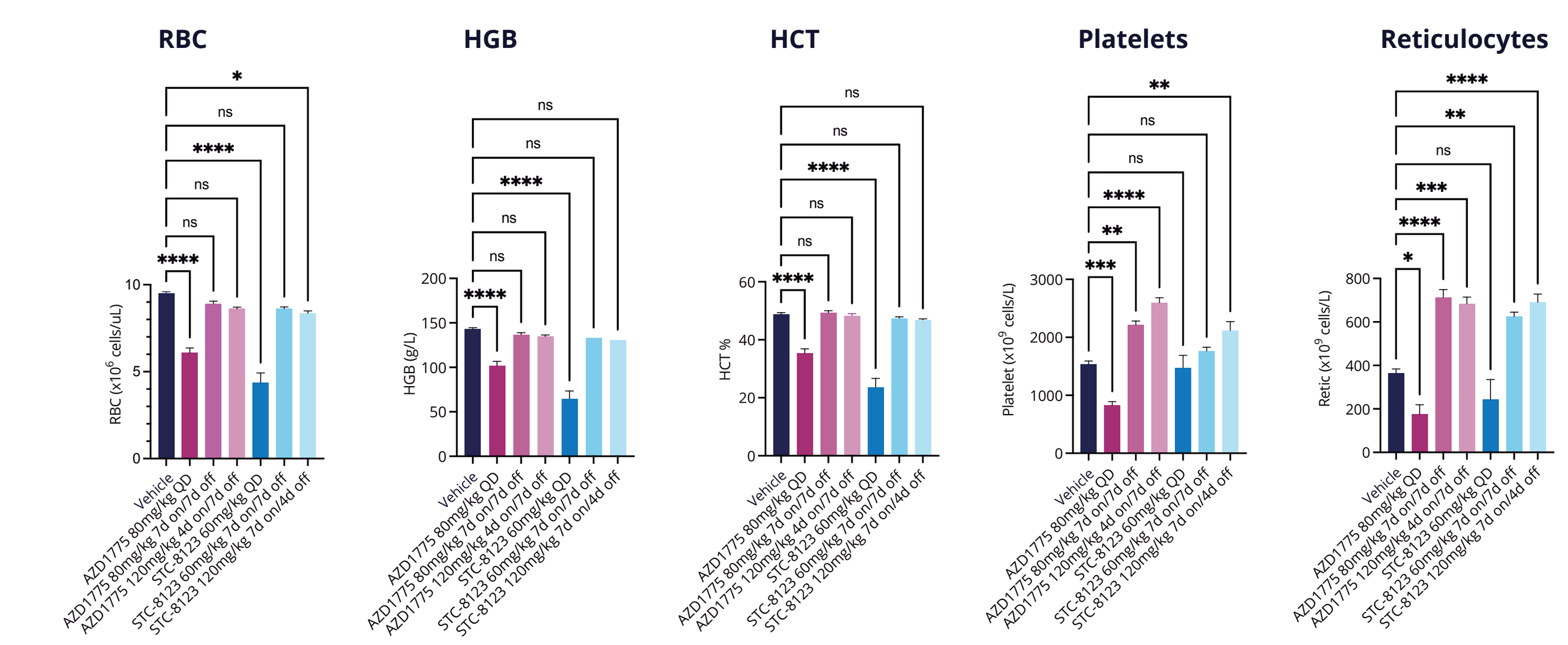


Compound Treatment with Various Dosing Schedules: Anti-tumor Activity and Tolerability in A427 Tumor Model



One way Anova test. *p<0.05; Mean ± SEM (n=6/group)

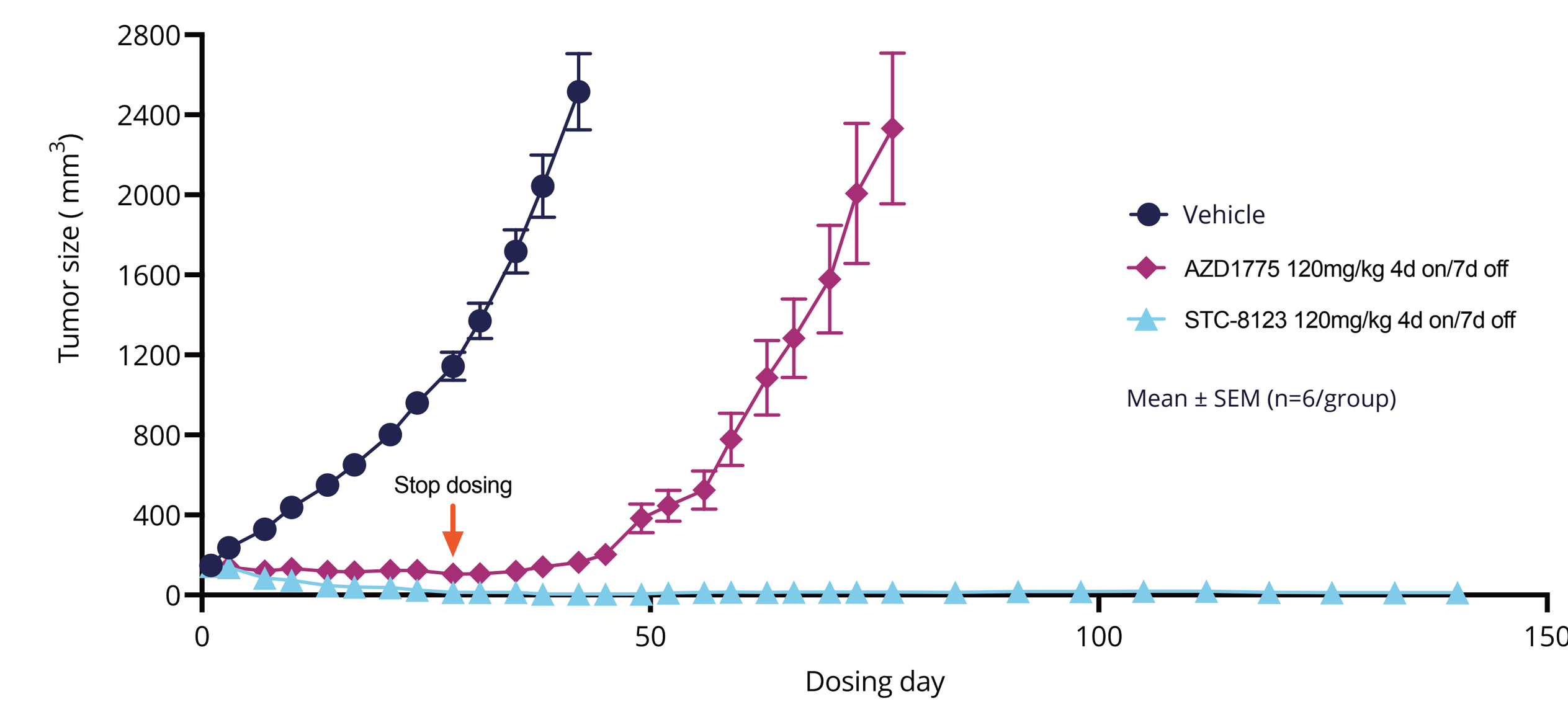
7-Day Dosing Holiday Allows Full Recovery of Blood Counts



One way Anova test. *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001; Mean ± SEM (n=6/group)

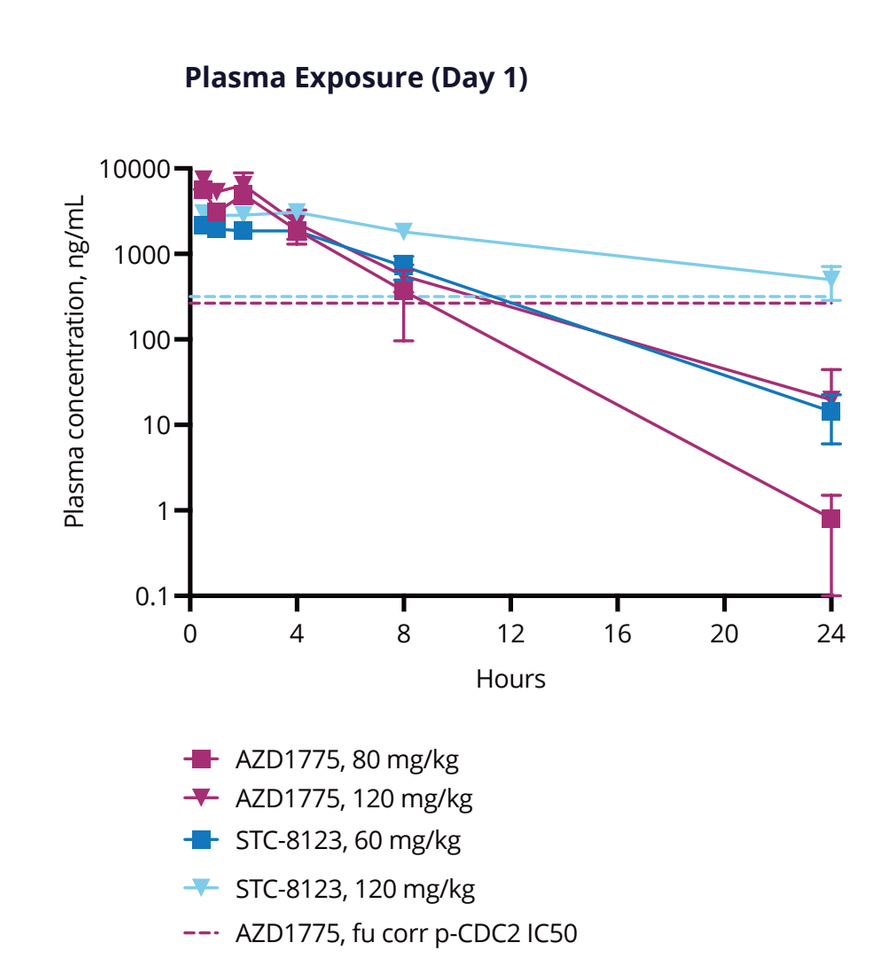
At least 200 uL of whole blood used for hematology analysis immediately after collection by ADVIA 2120 (Siemens): RBC (red blood cells), HGB (hemoglobin), HCT (hematocrit blood cell pressure), platelets, and reticulocytes.

No Tumor Regrowth Observed After STC-8123 High Dose Treatment Stopped in A427 Tumor Model



After 3 treatment cycles with dosing holidays, 120 mg/kg STC-8123 eliminates tumors in A427 tumor mice with no tumor regrowth observed after treatment termination. The same dose and schedule with AZD1775 results in tumor regrowth soon after treatment has been stopped.

Sustained PK and High Tumor Exposure May Contribute to Maintenance of Anti-Tumor Activity in Dosing Holidays



Tumor Exposure (Last Day)

Treatment, Dose, and Schedule		Tumor exposure, 8 hr (ug/g) Ave ± SEM
AZD1775	80 mg/kg, QD	Day 42: 4000 ± 1000 (n=5)
	80 mg/kg, 7d on/7d off	Day 42: 5 ± 3 (n=6)
	120 mg/kg, 4d on/7d off	Day 33: 3 ± 2 (n=5)
STC-8123	60 mg/kg, QD	Day 42: 62000 ± 11000 (n=5)
	60 mg/kg, 7d on/7d off	Day 42: 1200 ± 200 (n=6)
	120 mg/kg, 4d on/7d off	Day 33: 130 (n=1)

Conclusions

- Novel, potent, and orally available Wee1 inhibitors were identified through the application of Schrödinger's computational platform, including Free Energy Perturbation (FEP+) and Protein FEP+.
- Representative compounds from three series show superior kinase selectivity compared to AZD1775 and Zn-C3 in a broad kinase panel (ScanMAX; >450 kinases).
- STC-8123 shows desirable ADME properties and PK profiles in preclinical species. STC-8123 demonstrates robust anti-tumor activity and sustained target engagement in vivo in tumor models.
- Tumors are eradicated with high dose STC-8123 treatment and all treated mice remain tumor free after treatment for at least four months while tumors in AZD1775 treated mice grow back within one month of stopping treatment.
- The anti-tumor effects of STC-8123 are maintained during dosing holidays while allowing full recovery of mechanism-based hematological effects, likely due to its sustained plasma concentrations and high exposure in tumors.
- Advanced Wee1 program compounds show no detectable CYP3A4 time dependent inhibition while maintaining potency, selectivity, and anti-tumor activity. A development candidate is expected to be selected in 2022 and an IND submission is anticipated in 2023.

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