KYINNO Biotechnology

Drug Discovery Service

From BTK In-Situ Mutation (C481S, A428D, etc.) to In Vivo Screening: A Comprehensive Platform to Address Drug Resistance in BTK-targeted Therapy

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Abstract

Bruton's tyrosine kinase (BTK) is a core regulatory molecule in the B-cell receptor (BCR) signaling pathway. It is constitutively expressed in myeloid and lymphoid cells and plays a decisive role in the proliferation, survival, differentiation, activation, and apoptosis of B cells. Abnormal activation of BTK is closely associated with B-cell malignancies such as chronic lymphocytic leukemia (CLL) and mantle cell lymphoma (MCL). In hematologic tumors, BTK inhibitors block key steps in the BCR signaling pathway, thereby inhibiting the proliferation and survival of tumor cells .

Ibrutinib is the first effective covalent Bruton's tyrosine kinase inhibitor (BTKi), approved by the U.S. Food and Drug Administration (FDA) in 2013, ushering in an era of chemotherapy-free treatment for B-cell malignancies. Second-generation covalent BTK inhibitors, including acalabrutinib, zanubrutinib, and orelabrutinib, have also been approved for marketing in China. The advent of BTK inhibitors has had profound significance for the treatment of B-cell malignancies, improving the treatment modalities and prognosis for patients with B-cell lymphoma .

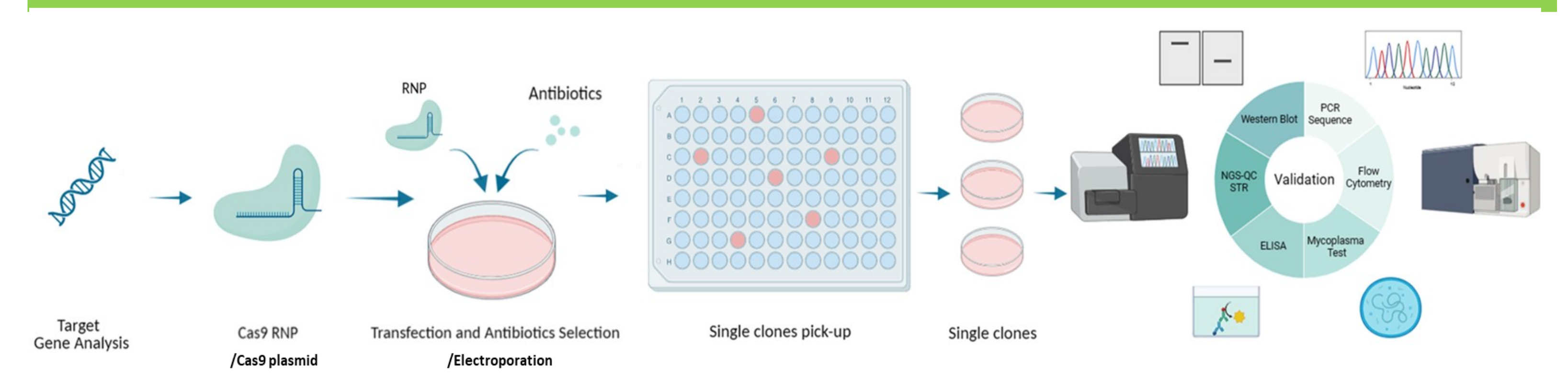
However, traditional BTK inhibitors exhibit poor kinase selectivity and a high incidence of off-target adverse reactions. Moreover, in recent years, although BTK inhibitors (such as ibrutinib and acalabrutinib) have significantly improved patient survival, the issue of drug resistance has become increasingly prominent—approximately 30% of patients develop resistance due to BTK gene mutations (e.g., C481S, T474I, L528W, etc.), limiting therapeutic efficacy .

Through breakthroughs in in-situ mutation technology, kyinno has precisely introduced specific BTK mutations directly into the genome of TMD8 cells, preserving the natural expression regulatory mechanisms. This provides a more clinically relevant "touchstone" for analyzing BTK resistance mechanisms and developing targeted therapies. Kyinno has developed over 20 BTK in-situ mutant cell lines (including single mutations such as C481S, T474I, A428D, L528W, and compound mutations). These novel cell models, based on in-situ mutation technology, enable systematic evaluation of the dynamic impact of different mutations on drug binding and guide the structural optimization of next-generation inhibitors. Additionally, we have developed in vitro and in vivo screening platforms based on these mutant cell lines, enabling rapid validation of lead compounds from the molecular to the organism level, thereby accelerating BTK inhibitor development .

TMD8 BTK Cell Panel

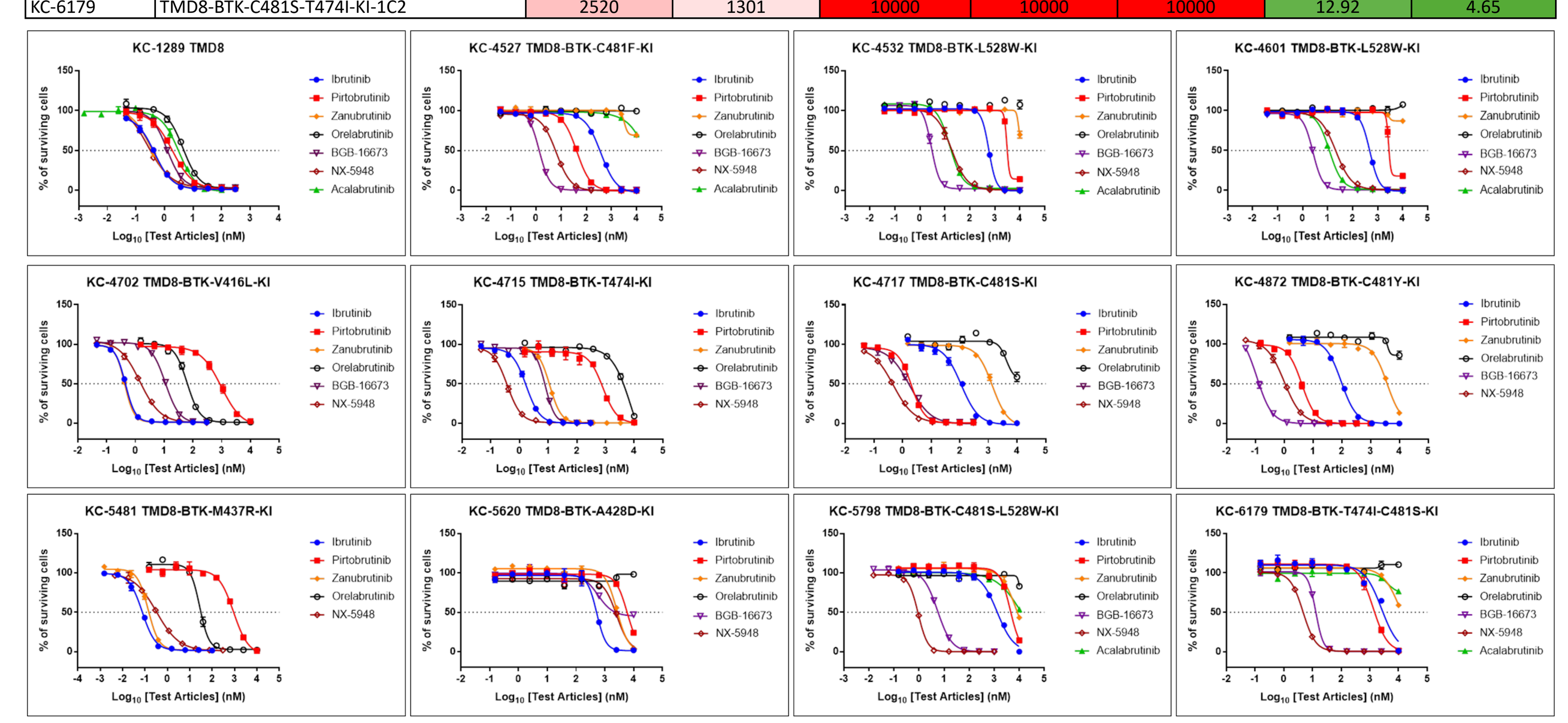
No.	ID	Cell Line name
1	KC-1289	TMD8
2	KC-4527	TMD8-BTK-C481F-KI-1#
3	KC-4528	TMD8-BTK-C481F-KI-3#
4	KC-4532	TMD8-BTK-L528W-KI 3#
5	KC-4601	TMD8-BTK-L528W-KI 1#
6	KC-4701	TMD8-BTK-V416L-KI-1B1
7	KC-4702	TMD8-BTK-V416L-KI-1A3
8	KC-4715	TMD8-BTK-T474I-KI-1A1
9	KC-4716	TMD8-BTK-T474I-KI-1C1
10	KC-4717	TMD8-BTK-C481S-KI-1B3
11	KC-4718	TMD8-BTK-C481S-KI-1C6
12	KC-4871	TMD8-BTK-C481Y-KI-1A2
13	KC-4872	TMD8-BTK-C481Y-KI-1B2
14	KC-5481	TMD8-BTK-M437R-KI-1A1
15	KC-5482	TMD8-BTK-M437R-KI-1C1
16	KC-5619	TMD8-BTK-A428D-KI-1A1
17	KC-5620	TMD8-BTK-A428D-KI-1A2
18	KC-5798	TMD8-BTK-C481S-L528W-KI-1A3
19	KC-5799	TMD8-BTK-C481S-L528W-KI-1A5
20	KC-6178	TMD8-BTK-C481S-T474I-KI-1B1
21	KC-6179	TMD8-BTK-C481S-T474I-KI-1C2
22	KC-5107	TMD8-CRBN-KO-1A3
23	KC-4700	TMD8-BTK-HiBiT

Schematic Diagram & Flowchart for Cell Line



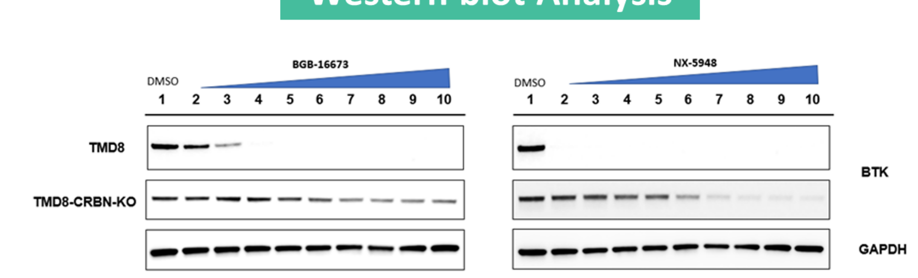
In Vitro Drug Activity and IC_{50} Summary

Cell ID	Cell line	Compound IC_{50}(nM)						
		Ibrutinib	Pirtobrutinib	Zanubrutinib	Orelabrutinib	Acalabrutinib	BGB-16673	NX-5948
KC-1289	TMD8	0.41	1.88	0.37	4.77	4.44	1.27	0.32
KC-4527	TMD8-BTK-C481F-KI-1#	380.3	41.9	10000	10000	10000	1.32	6.34
KC-4528	TMD8-BTK-C481F-KI-3#	286.2	28.76	10000	10000	10000	0.83	2.77
KC-4532	TMD8-BTK-L528W-KI 3#	486.3	2722	10000	10000	11.73	2.55	21.25
KC-4601	TMD8-BTK-L528W-KI 1#	574.3	3030	10000	10000	15.75	2.87	18.63
KC-4701	TMD8-BTK-V416L-KI-1B1	1	650.78	1	52.88	TBD	4.92	0.64
KC-4702	TMD8-BTK-V416L-KI-1A3	0.66	855.56	1	64.21	69.17	11.24	1.57
KC-4715	TMD8-BTK-T474I-KI-1A1	2.33	714.22	10.91	4191.92	36.57	7.61	0.36
KC-4716	TMD8-BTK-T474I-KI-1C1	2.87	778.85	16.55	3145.66	TBD	3.71	0.62
KC-4717	TMD8-BTK-C481S-KI-1B3	108.8	2.47	1299.03	10000	10000	1.65	0.45
KC-4718	TMD8-BTK-C481S-KI-1C6	257.78	5.22	2805.91	10000	TBD	6.01	1.11
KC-4871	TMD8-BTK-C481Y-KI-1A2	124.3	3.24	4001	3619	10000	0.21	0.7
KC-4872	TMD8-BTK-C481Y-KI-1B2	95.06	4.09	3733	10000	TBD	0.11	1
KC-5481	TMD8-BTK-M437R-KI-1A1	0.08	908.7	0.14	27.93	TBD	TBD	0.33
KC-5482	TMD8-BTK-M437R-KI-1C1	0.12	1175	0.17	24.74	TBD	TBD	0.54
KC-5619	TMD8-BTK-A428D-KI-1A1	438	5400	2206	10000	10000	576.8	1991
KC-5620	TMD8-BTK-A428D-KI-1A2	520.6	5998	2602	10000	10000	562.9	2549
KC-5798	TMD8-BTK-C481S-L528W-KI-1A3	1309	4351	7797	10000	10000	5.49	0.92
KC-5799	TMD8-BTK-C481S-L528W-KI-1A5	1432	4285	6118	10000	10000	3.54	0.75
KC-6178	TMD8-BTK-C481S-T474I-KI-1B1	3035	1561	10000	10000	10000	15.33	5.8
KC-6179	TMD8-BTK-C481S-T474I-KI-1C2	2520	1301	10000	10000	10000	12.92	4.65

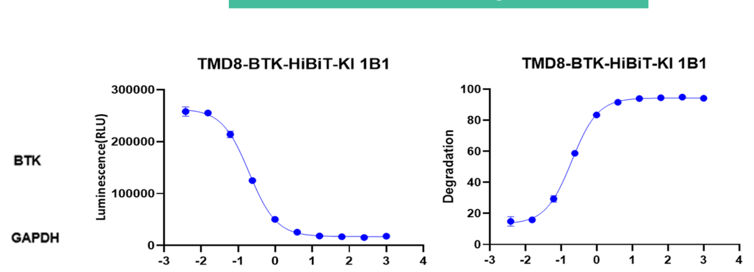


PROTAC Degradation in TMD8-BTK Cells

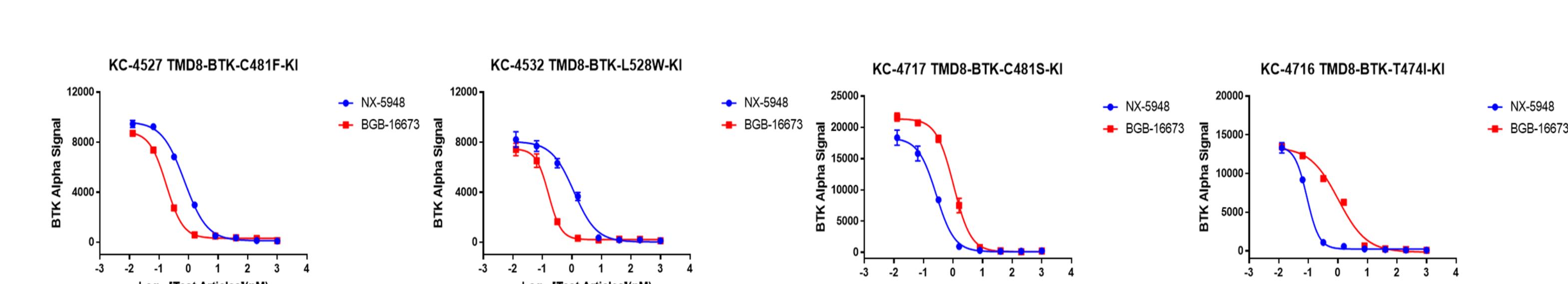
Western blot Analysis



Hibit Analysis

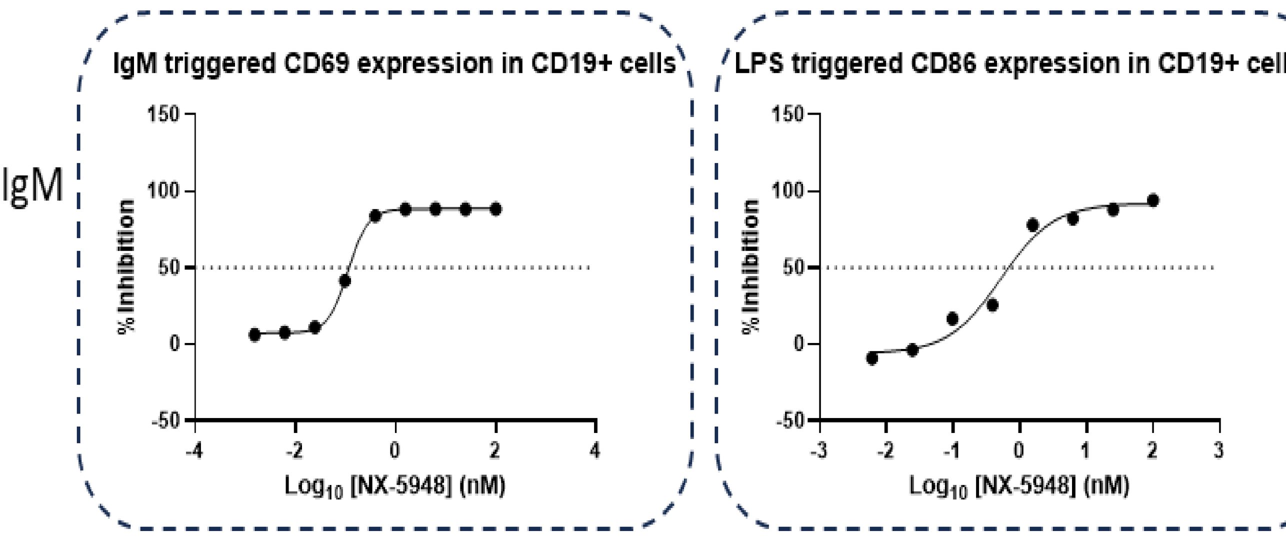


AlphaLISA Analysis



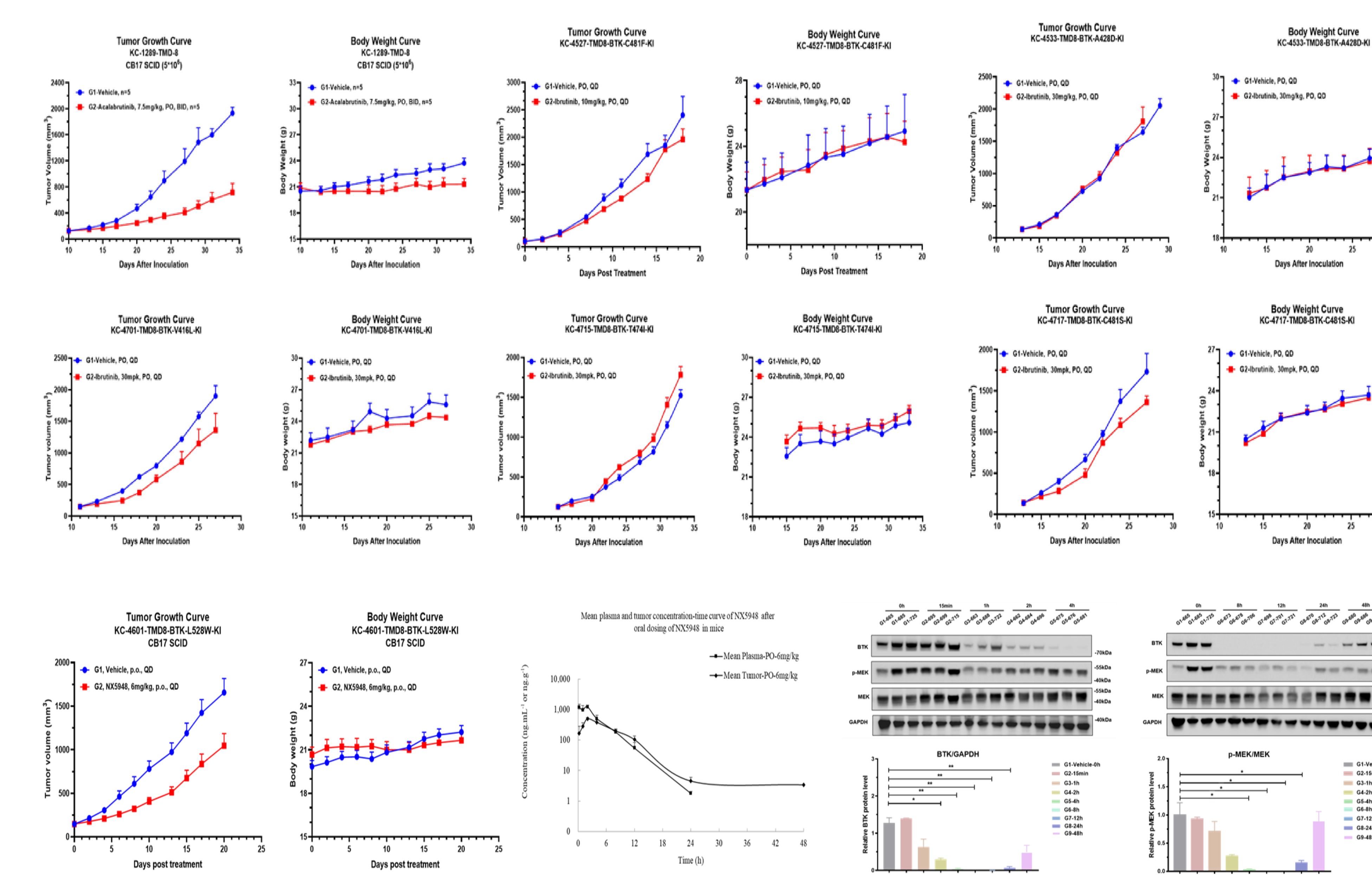
Immune Assay

Stimulator: IgM
Cell: PBMC



Stimulator: LPS
Cell: PBMC

In Vivo Efficacy



Summary

- Over 20 TMD8-BTK gene-edited cell lines that cover most hot mutations have been constructed, such as C481S, C481F, L528W, V416L, T474I and A428D mutation.
- TMD8-BTK gene-edited cell lines have been well validated through sequencing, and *in vitro/in vivo* efficacy testing.
- TMD8-BTK gene-edited cell lines constitute a comprehensive platform for addressing drug resistance in BTK-targeted therapy.

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