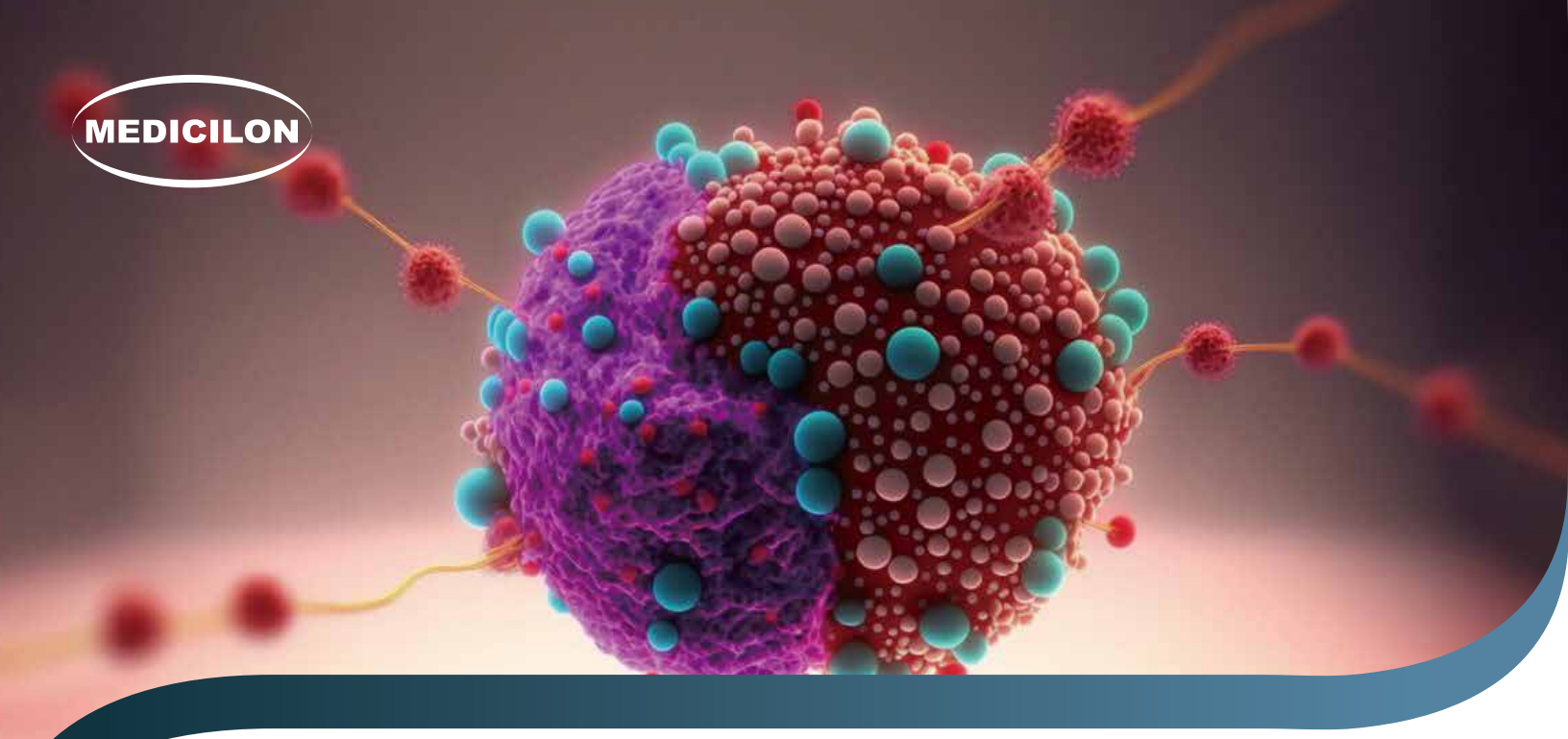


Medicilon Drug Resistant Tumor Models

Cancer is a leading cause of deaths across the globe. About 20 million people are diagnosed with cancer every year. As age of population increases, the incidence rates of cancer climb steadily in recent years. The most common types of cancer are breast cancer, lung cancer and gastrointestinal cancer.

Great progresses in cancer biology and cancer therapy have been witnessed in the past few decades, bringing new hopes to cancer patients. With the application of targeted therapies, immune therapies, cell therapies and other advanced treatments, the survival of patients has been prolonged significantly, and the quality of patients' lives has also been improved greatly. Nowadays, cancer tends to be managed as a chronic illness instead of a life-threatening disease, and this highlights the importance of drug resistance issues observed in tumors.

Drug Resistance of Tumors

Drug resistance of tumors refers to the phenomenon where tumor cells in the patient's body develop higher tolerability to the original therapy post a period of treatment, leading to a compromised therapeutic benefit and disease progression. The development of resistance may be due to the presence of pre-existing tumor cell populations which are not responsive to the original treatment. These populations can survive from the treatment and proliferate, leading to recurrence. It is also possible that, during the treatment process, tumor cells acquire the resistance out of genomic alternation, epigenetic changes, metabolism and immune regulations, etc.

The occurrence of drug resistance is both universal and individualized. Specifically, resistance can potentially arise for various types of drugs or treatments, and the underlying mechanisms may also vary from patient to patient.

According to the researches, mechanisms of resistance development include but are not limited to the following aspects: (1) variation on the molecular structures of target molecules on cancer cells, which leads to inefficient binding between drug molecules and the target protein; (2) activation of alternative pathways to bypass the pathway that is targeted by the treatment; (3) harnessing the cell survival and metastasis abilitiesvia genetic and/or epigenetic regulations (e.g. gene repair, invasion, etc.) to acquire higher tolerability to the treatment; (4) production of specific metabolites to neutralize or deactivate the drug components; (5) modulation of intracellular and extracellular metabolic pathways (e.g. influx and efflux) or formation of sheath (e.g. extracellular matrix) to limit the access of drug components to cancer cells.

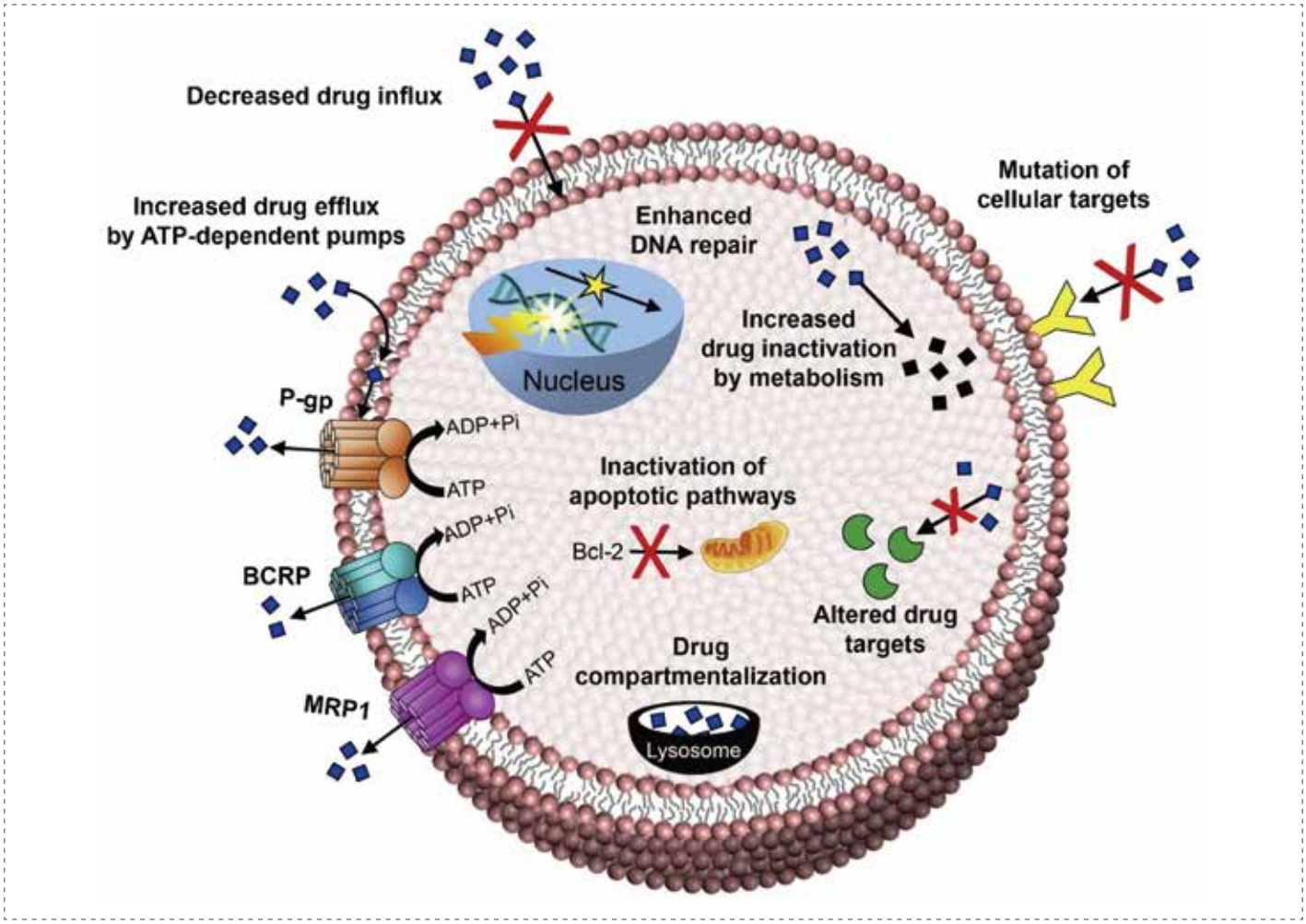


Figure 1. Mechanisms of Drug Resistance[1]

Development of Resistant Models in Medicilon

Resistant models are important preclinical research tools for drug resistance mechanism study and development of novel therapeutic drugs or strategies. In Medicilon, we exploit drug exposure induction approach for drug resistant model development purpose. The following 4 drugs are selected for resistant model development: Herceptin, Osimertinib, Abemaciclib and Sotorasib.

Introduction to the selected drugs

Herceptin

Trastuzumab (Brand name: Herceptin®) is a HER2/neu receptor antagonist developed by Genentech. It was first approved by the FDA in 1998 for treating patients with HER2 overexpressing breast cancer, gastric cancer and gastroesophageal junction adenocarcinoma. It is usually combined with taxel. It is the first approved TKI, which created the paradigm for biomarker-driven drug discovery and development.

Osimertinib

Osimertinib (Brand name: TAGRISSO®) is a third-generation, irreversible EGFR-TKI developed by AstraZeneca. It was approved by the FDA in 2015 for treating patients with locally advanced or metastatic EGFRm NSCLC, typically with EGFR ^{T790M} mutation feature.

Abemaciclib

Abemaciclib (Brand name: Verzenio®) is a CDK4/6 inhibitor developed by Eli Lilly. It was approved by the FDA in 2017 for treating patients with HR^{+}/$HER2^{-}$ advanced or metastatic breast cancer that has progressed after taking therapy that alters a patient's hormones (endocrine therapy).

Sotorasib

Sotorasib or AMG510 (Brand name: LUMAKRAS®) is a RAS GTPase inhibitor developed by Amgen. It was granted with accelerated approval by the FDA in 2021 for treating patients with KRAS ^{G12C}-mutated locally advanced or metastatic NSCLC. Beforehand, KRAS ^{G12C} mutation was regarded as an undruggable target in cancer. In December 2023, since the PFS data from the CodeBreaK 200 trial (NCT04303780) could not be reliably interpreted, the FDA has rejected the sNDA for Lumakras.

Strategies for Resistant Model Development

To develop drug resistant models, we exploit a strategy of using drug exposure for a period of time to induce the resistance development on cancer cell lines. Specifically, we combined short-term, pulsed drug exposure with continuous drug exposure to induce the resistance. The dosing pulse at a higher concentration is given at 4~6 hours per dose and a couple of days are given for cells to recover between 2 continuous pulses. This setting aims to better mimic the drug metabolism and exposure patterns in real patients in clinic. To ensure that the induced resistance out of pulsed dosing is not lost, cells will be exposed to a continuous dosing of drug at a relatively low concentration. After passing a sufficient number of dosing cycles, the resistant type of cells will be compared with the parental one in terms of the sensitivity to the therapeutic compound. We assess the IC_{50} values for both resistant and parental lines and calculate the ratio (i.e. RF value), which will be used to evaluate how strong the resistance has been acquired out of the process and determine whether a resistant cell line model has been successfully developed. Referring to McDermott et al [2], we set a reversal fold (RF) value > 5 as the criteria of resistant model development.

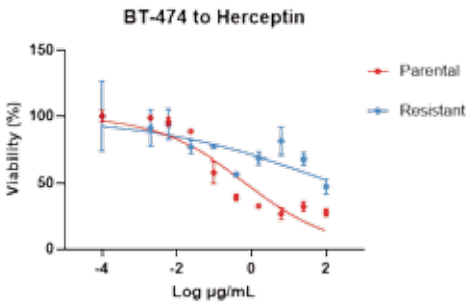
Upon successfully developing a resistant cell line model, we will further establish the xenograft model on immuno-deficient mice to evaluate the tumor formation of resistant cell line as well as assess its tolerability to the drug with a comparison of the parental cell line-derived tumor model.

Our Results

Herceptin

To develop a Herceptin-resistant model, we first chose BT-474, an ER$^+$/HER2$^+$ human breast cancer cell line. Through a period time of drug exposure, we succeeded in developing the Herceptin-resistant cell line. As shown in Figure 2a, the IC_{50} value of parental cell line was 0.693 μg/mL, while the IC_{50} of resistant cell line was escalated to 174.5 μg/mL, giving an RF value of 251.8. In the *in vivo* efficacy study, the %TGI values of parental and resistant models by Herceptin were 85.11 and 52.57 (Figure 2b).

a

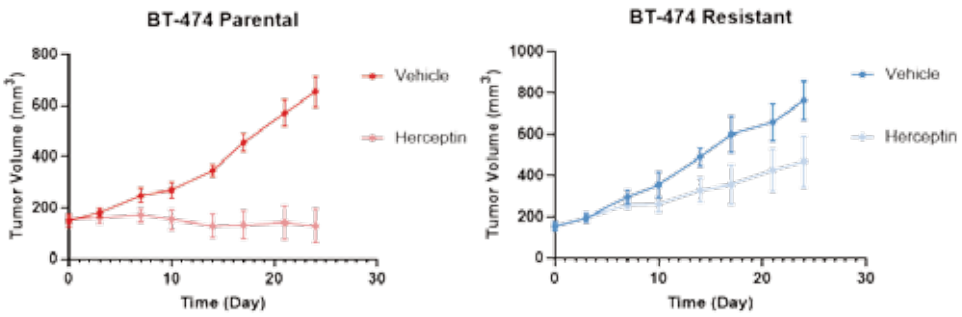


***In vitro* Drug Screening**

	Parental	Resistant
IC_{50} (μg/mL)	0.6930	174.5
HillSlope	-0.3668	-0.1737

RF=251.8

b



***In vivo* Drug Efficacy**

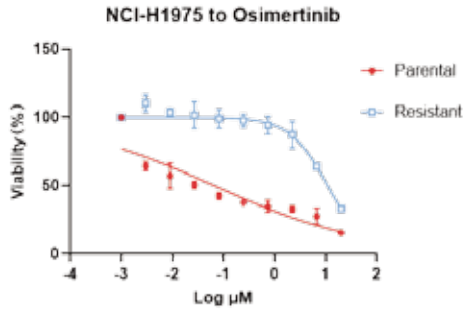
	Parental (Day 24)	Resistant (Day 24)
%TGI	85.11	52.57
%T/C	11.74	48.58

Figure 2. BT-474_Herceptin Resistant Model Experimental Results:
a) *in vitro* drug sensitivity study using CCK-8 assay; b) *in vivo* tumor formation and efficacy study.

Osimertinib

For Osimertinib-resistant model development purpose, we chose NCI-H1975, a human NSCLC cell line with EGFR $^{L858R/T790M}$ mutation features. Through drug exposure-mediated induction process, we succeeded in obtaining a resistant cell line. As shown in Figure 3a, the IC_{50} value of parental cell line was 65.9 nM, while the IC_{50} of resistant cell line was escalated to 10940 nM, giving an RF value of 166. In the *in vivo* efficacy study, the tumor suppression by Osimertinib was evaluated by tumor growth inhibition rate or %TGI. The %TGI of parental cell line-derived xenograft model to Osimertinib was determined to be 93.72, while that of resistant cell line-derived one dropped to 70.05 (Figure 3b).

a

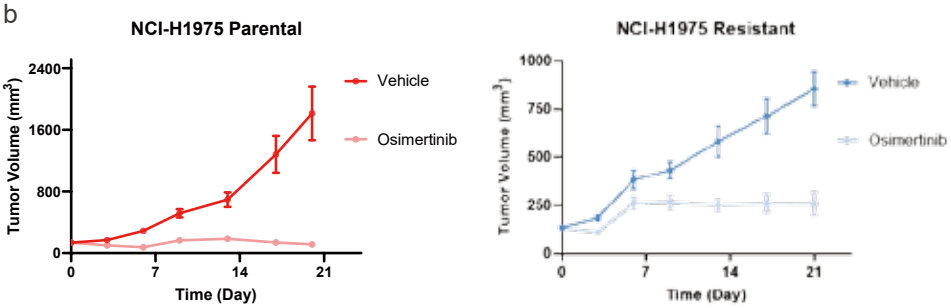


***In vitro* Drug Screening**

	Parental	Resistant
IC_{50} (nM)	65.9	10940
HillSlope	-0.2896	-1.161

RF=166

b



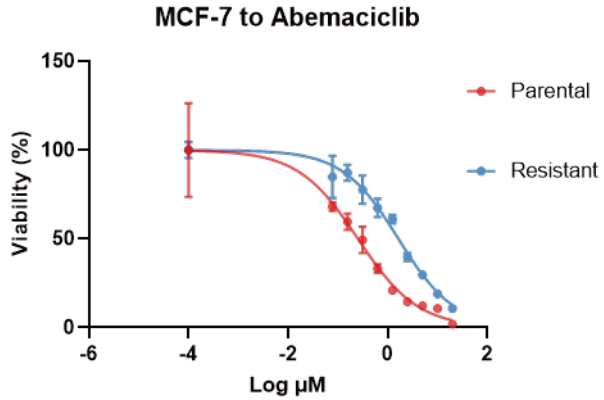
***In vivo* Drug Efficacy**

	Parental (Day 20)	Resistant (Day 21)
%TGI	93.72	70.05
%T/C	6.22	28.11

Figure 3. NCI-H1975_Osimertinib Resistant Model Experimental Results:
a) *in vitro* drug sensitivity study using CCK-8 assay; b) *in vivo* tumor formation and efficacy study.

Abemaciclib

To develop an Abemaciclib-resistant model, we chose MCF-7, a $HER2^-$ human breast cancer cell line. Through a period time of drug exposure, we succeeded in developing the Abemaciclib-resistant cell line. As shown in Figure 4, the IC_{50} value of parental cell line was 0.2536 μM, while the IC_{50} of resistant cell line was escalated to 1.649 μM, giving an RF value of 6.5. Given that the MCF-7 xenograft model had some tumor formation issue, the in *vivo* results are not provided here.



***In vitro* Drug Screening**

	Parental	Resistant
IC_{50} (μM)	0.2536	1.649
HillSlope	-0.7205	-0.7709

RF=6.5

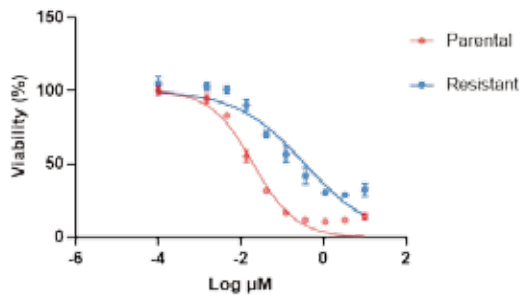
Figure 4. *In Vitro* Drug Sensitivity Study of MCF-7_Abemaciclib-Resistant Cell Line Model, CCK-8 assay.

Sotorasib (AMG510)

To develop a Sotorasib-resistant model, we chose NCI-H358, a human NSCLC cell line with KRAS ^{G12C} mutation feature. Through a period time of drug exposure, we succeeded in developing the resistant cell line. As shown in Figure 5a, the IC_{50} value of parental cell line was 19.99 nM, while the IC_{50} of resistant cell line was escalated to 350.2 nM, giving an RF value of 17.52. The *in vivo* study of NCI-H358 is in progress and the results are not available for disclosing yet.

In a separate study, we successfully induced resistance to Sotorasib on a Calu1 human lung cancer xenograft model via continuous dosing approach, as shown in Figure 5b.

a

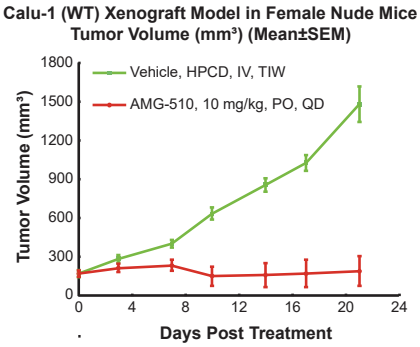


***In vitro* Drug Screening**

	Parental	Resistant
IC_{50} (nM)	19.99	350.2
HillSlope	-0.8440	-0.5254

RF=17.52

b



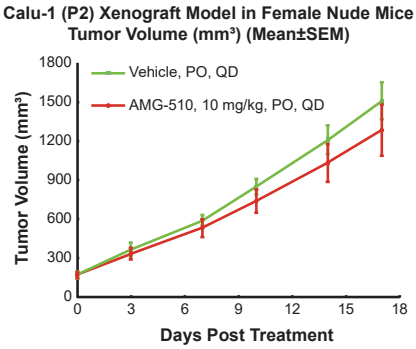


Figure 5. Sotorasib-Resistant Model Development Results:

a) *in vitro* drug sensitivity study of NCI-H358_Sotorasib resistant model, CCK-8 assay; b) *in vivo* tumor efficacy study of Calu1 xenograft model.

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[1] Vrinda Gote, et al. Drug Resistance in Metastatic Breast Cancer: Tumor Targeted Nanomedicine to the Rescue. Int J Mol Sci. 2021 Apr 28;22(9):4673. doi: 10.3390/ijms22094673.

[2] Martina McDermott, et al. In vitro Development of Chemotherapy and Targeted Therapy Drug-Resistant Cancer Cell Lines: A Practical Guide with Case Studies. Front Oncol. 2014 Mar 6:4:40. doi: 10.3389/fonc.2014.00040.

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