Review

Discovery Medicine 2025; 37(201): 2135–2144
https://doi.org/10.24976/Discov.Med.202537201.182

Real-World Lung Cancer Cell Lines for Preclinical Development and Testing of Therapeutics

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Submitted: 11 July 2025 Revised: 14 August 2025 Accepted: 9 September 2025 Published: 20 October 2025

In preclinical drug development, the success rate of carrying candidates from phase I trials to final approval is as low as ~10%. Initially, compounds hitting specific molecular targets are selected via testing on permanent cell lines, xenotransplants, and animal experimentation. However, numerous failed clinical trials demonstrate that these models lack sufficient predictive power. In contrast to established cell lines altered by extended periods in tissue culture, freshly isolated cancer cells may represent original characteristics. For typical solid tumors, access to representative tumor cells is difficult, although pleura-derived tumor cells of advanced lung cancer patients may provide a notable exception to obtain original cell suspensions. These lung cancer cells can be used directly or upon the establishment of cell lines, which can be obtained rapidly at a high take rate due to the aggressive nature of these tumors. In contrast to established permanent cell lines, freshly isolated cells exhibit higher resistance and may more accurately predict responses. Furthermore, these cell lines can be validated within the framework of functional precision medicine for personalized chemotherapy by comparing the *in vitro* chemosensitivity profile of cells to clinical responses for specific inhibitors directed at mutated drivers such as Kirsten rat sarcoma viral oncogene homolog (KRAS), epidermal growth factor receptor (EGFR), anaplastic lymphoma kinase (ALK), c-ros oncogene 1, receptor tyrosine kinase (ROS1), and neurotrophic tropomyosin receptor kinase (NTRK) oncogenes in non-small cell lung cancer (NSCLC). This review discusses the use of freshly cultivated lung cancer cells from pleural effusions and highlights their value in generating clinically relevant predictive data, in contrast to permanent cell lines.

Keywords: non-small cell lung cancer; cancer precision medicine; pleural effusion; chemosensitivity test

Introduction

The emergence of targeted precision medicine has led to extensive development and screening of large drug libraries aimed at proteins implicated in the progression of cancer and other diseases. Success rates in late-stage drug development from phase III trials to launch have increased from under one in two a decade ago to almost two in three between 2015–2017, across all indications, and particularly for rare diseases [1]. Phase II success rates have remained relatively constant, with approximately a quarter of candidates reaching phase III after phase II trials. About 12% of drug candidates passing preclinical studies enter clinical trials. The probability of approval from the beginning of phase I has remained stable at less than 10%, with 80% of withdrawals attributable to safety or efficacy reasons [2,3]. The average first approval rate across pharmaceutical companies is 14.3%, with values ranging from 8% to 23% [4]. The time span from a novel therapeutic approach to the approval of a new drug averages 14 years. Therefore, early clinical development determines research and development efficiency and represents a bottleneck in drug development. It seems obvious that cancer cell lines and experimental animal models used in drug selection partially lack predictive power for clinical efficacy. This review emphasizes the use of freshly established lung cancer cell lines for preclinical testing, as they provide results with greater predictive power compared to permanent cancer cell lines maintained in culture for decades.

Identifying Cancer Cell Lines as Proxies for Patient Tumors

Cancer cell lines are employed as a model for patient tumor samples to investigate the efficacy of drugs. Cell lines are inexpensive, and they can be cultured for long periods of time, allowing repetitions of tests [5]. The establishment of a new cell line is regarded as complex with a low success rate, as well as time-consuming, often requiring more than one or two years [6,7]. However, *in vitro* culture of cell lines alters their characteristics, such as growth rate and chemosensitivity, resulting in severe aberrations from the original tumor profile [8–10]. Genetic changes, as well as altered mRNA expression, have been described for cell lines in tissue culture [11–13]. It is commonly believed that this problem is partially solved by a

similarity score that maps genomic datasets of cell lines to those of patient tumor samples. Extended parameters include multi-omics results for gene expression, mutations, copy number aberration (CNA), DNA methylation, and microRNA expression [10,14,15]. Multi-omics profiles of tumor samples are available from the Cancer Genome Atlas (TCGA) [10,16] and the Cancer Cell Line Encyclopedia (CCLE) [14]. Liu *et al*. [17] used a Transcriptome Correlation (TC) analysis to compare cell lines and tumor samples, and Warren *et al*. [18] developed an alignment method called Celligner for this mapping. Several high-ranked cell lines recapitulated the drug response of patient samples for several FDA-approved cancer drugs. However, next-generation sequencing (NGS) profiles from 947 human cancer cell lines, coupled with drug responses for 24 anticancer drugs across 479 cell lines, showed that genetic characteristics varied substantially between different cancers, even if they stemmed from the same tissue [19,20]. It is therefore not feasible for either NCI cell line–based or typical xenograft-based models to reliably predict human clinical response for a particular type of cancer [9].

Typically, alignment of cell lines with tumor samples of the same cancer type may show concordance for some lines while revealing systematic differences in others [5,21]. Based on similarity scores, several high-ranking cell lines were shown to closely resemble the correct tumor type in previously published *in vitro* tests [8]. For lung cancer adenocarcinoma, the cell lines HCC4006, NCI-H222, NCI-H2291, NCI-H3122, and RERFLCK exhibited high similarity to tumors; however, they were used infrequently in experiments. Cell lines lacking characteristics of actual tumor cells can exaggerate screening results of drugs with respect to higher chemosensitivity and lead to a loss of predictive power for clinical outcomes. Panels of cell lines with defined mutations are available for controlled *in vitro* experiments, such as those from the National Cancer Institute [22]. Cell lines need to be adapted to specific culture conditions, and this favors clone selection, thus limiting cell heterogeneity. It is not always easy to generate cancer cell lines, and their ability to proliferate indefinitely may cause progressive changes in gene expression profiles [23]. However, immortalized cell lines with long-term cultivation are not always predictive of actual cancer behavior [9,24].

Primary Cell Culture Lines

Reliance on data from experimental animal research to select drugs for clinical trials has yielded a high failure rate of 95% for targeted agents in early-phase trials [25]. Often, promising preliminary results of preclinical experiments do not translate well to clinical settings [26,27]. Progressing from two-dimensional cell culture to three-dimensional cell models, such as spheroids, recapitulates some features of spatial tumor architecture yet fails to incorporate tumor vasculature and microenvironment. Patient-derived xenografts (PDX) are hindered by low engraftment rates and slow growth in comparison to cells from primary tumor material, and furthermore, the stroma and vessels supporting their growth are of murine origin [28].

Primary cancer cell cultures are *ex vivo* cell populations isolated directly from surgically resected tumor tissues or biopsies [29]. Following tissue dissociation by chemical, mechanical, and enzymatic processes, the resulting cell suspensions may comprise a mixture of subclones of the whole tumor mass. Primary cell culture is a gold-standard testing platform for *in vitro* research in oncology, as it reflects the tumor state more accurately compared to commonly employed cell lines [30,31]. Provided that primary cells are directly derived from the tissue of origin and cultured under favorable conditions, they are more representative of the *in vivo* state. The lifespan of these patient-derived primary cells is regarded as limited to a few passages. Moreover, the success rate of primary cell line establishment has been considered quite low and, thus, extensive biochemical, histological, and genomic characterization needs to be conducted [32]. The low availability of representative tumor tissues, their content of distinct tumor clones with different chemosensitivities in low numbers, and the difficulties of harvesting single-cell suspensions for *in vitro* expansion hamper the development of predictive drug tests for clinical application.

Lung Cancer Malignant Pleural Effusions

Despite costly target validation and drug optimization in preclinical models, most therapies still fail in phase III clinical trials [33]. The underlying cause is that cell lines and xenografts that are commonly used are inadequate models and do not effectively mimic and predict clinical responses. A notable exception to the difficulties of obtaining tumor cells from surgical specimens is the procurement of cells from malignant pleural effusions (MPEs) [34,35]. MPEs accumulate due to an increase in pleural fluid production or inhibition of reabsorption by lymphatic obstructions caused by malignant tumors, increased microvascular permeability, and increased capillary venous pressure [36]. Pleural effusions hamper the ability of patients to breathe properly, and therefore, the excess fluid is drained by pleurocentesis to ease respiration. An accumulation of malignant pleural effusion is often the first sign of lung cancer and, in contrast to biopsies, malignant cells within the MPE are easily accessible and often highly abundant. These cells provide an excellent source of viable cells for molecular profiling, biomarker analysis, and establishment of primary cell cultures to study chemosensitivity. MPEs occur in about half of non-small cell lung cancer (NSCLC) patients at advanced stages and in most cases represent highly aggressive cancers with poor prognosis. Therefore, cells can be directly used for chemosensitivity testing and frequently give rise to permanent cell lines. The success rate

for the establishment of cancer cell lines has been described as low and unpredictable for any specimen of tumor tissue [16,37]. Kodack *et al*. [38] reported the success rate for establishing primary cancer cells from 568 patient tissues for a variety of tumor types and sources. This study found a success rate of 26%, most of them lung tumors, while pleural effusions had a higher rate (42%) compared to core biopsies (23%). For breast cancer, the take rate was low, with a success rate of 15%. In a study by Kim *et al*. [39], 23 patient-derived cell lines were isolated from 96 malignant effusions collected from 77 patients with advanced lung adenocarcinoma. In contrast, permanent lung cancer cell lines (estimated at 300–400), currently widely used, were established decades ago by Gazdar *et al*. [40] and other groups. Thus, the availability of freshly isolated pleura-derived lung cancer cells offers a unique possibility to guide precision medicine therapy for the respective patients by assessing their oncogenic drivers and corresponding inhibitors. In most cases, these effusions contain a high number of tumor cells in suspension that are enriched for aggressive tumor subpopulations, resulting in lower survival for such patients with so-called wet NSCLC [41].

Lung Cancer Therapy

The 5-year relative survival rates of lung cancer vary between 10% and 21% in Europe [42]. NSCLC accounts for around 85% of all lung cancer cases, while more than half of all new NSCLC cases are diagnosed at an advanced stage [43]. Systemic therapy used to consist of cytotoxic chemotherapy with only minor improvement in median overall survival [44]. Today, actionable biomarkers of NSCLC make this disease a model for precision oncology, comprising genomic alterations in epidermal growth factor receptor (EGFR) and those in Kirsten rat sarcoma viral oncogene homolog (KRAS), Erb-B2 receptor tyrosine kinase 2 (ERBB2), anaplastic lymphoma kinase (ALK), c-ros oncogene 1, receptor tyrosine kinase (ROS1), MET proto-oncogene, receptor tyrosine kinase (MET) and others [45,46]. KRAS mutations occur in up to 20–30% of patients with NSCLC and represent the most common oncogenic driver [47]. Adagrasib (MRTX849/Krazati) and sotorasib (AMG510/Lumakras) are both approved KRAS G12C inhibitors blocking this specific mutant form of the KRAS protein, which constitutes a frequent oncogenic driver in NSCLC [48]. In preclinical development, the KRAS G12C permanent cell lines NCI-H358 and NCI-H23 were sensitive to AMG-510 treatment with inhibitory concentration IC_{30} values of 35 nM in NCI-H358 and 44 nM in NCI-H23, indicating high cytotoxicity [48,49]. The IC_{50} for MRTX849 was 14 nM for NCI-H358 and 5 nM for MIA PaCa-2, respectively [50]. Despite these high toxic activities determined in *in vitro* studies, the clinical administration of AMG-510 and MRTX849 produced response rates of 40–50% with limited duration in patients pre-treated with chemo- or immunotherapy [47,51]. Several other KRAS G12C-specific therapies and pan-KRAS inhibitors are under development [52]. Driver mutations are detected using genomic tests that identify alterations in the respective genes through sequencing, most often via a limited panel of known drivers using NGS or whole-exome sequencing [53]. Specimens available for genomic analysis include biopsies, pleural effusions, ascitic fluid, circulating tumor DNA, and circulating tumor cells [54].

Functional Precision Cancer Medicine

Functional precision medicine (FPM) is a new approach that can guide cancer treatment by obtaining information from direct exposure of tumor-derived living cells to the respective drugs and assessment of their chemosensitivity [55]. Thereby, FPM supports clinical decisions on personalized treatment of cancer patients by functional instead of static genomic tests on tumor cells (Fig. 1). The first attempts to determine the chemosensitivity of *ex vivo* tumor cells were started more than four decades ago, but functional assays were too unreliable for clinical implementation, partially due to very low rates of tumor cell cultures [56–59]. Today, hematological tumors can be regularly tested, and prospective clinical trials have validated the predicted treatment response [60,61]. However, for solid tumors, the isolation of relevant tumor cells, expansion to cell numbers required for testing, and valid cytotoxicity testing are highly demanding and may fail for many patients; thus, only retrospective trials are recommended in solid tumors. Prolonged expansion of tumor cells may be necessary to obtain the minimum number required for reliable chemosensitivity testing. The isolated cancer cells are treated *in vitro* with different concentrations of chemotherapeutics to obtain IC_{50} values from dose–response curves [62]. The chemosensitivity results can be compared to the drug's peak plasma concentration to check whether effective concentrations may be reached *in vivo* [63]. Ultimately, a firm relationship between chemosensitivity data and clinical responses needs to be obtained and used for calibration of clinical trials. The predictive values of drug tests in freshly isolated tumor cells corroborate the use of these cell lines in preclinical drug development.

FPM in Lung Adenocarcinoma

We have previously reported the implementation of FPM for ALK-rearranged lung cancer [34]. These cells float freely in suspension and are not dependent on structured tumor–stroma relations, and can therefore be used for chemosensitivity tests without further processing. As a special advantage, the use of pleura-derived NSCLC cells is not hampered by the obstacles involved in the processing and expansion of solid tumor tissues [64,65]. The chemosensitivity of NSCLC lines isolated from pleural effusions can be tested within 4 days in *in vitro* cultures. Furthermore,

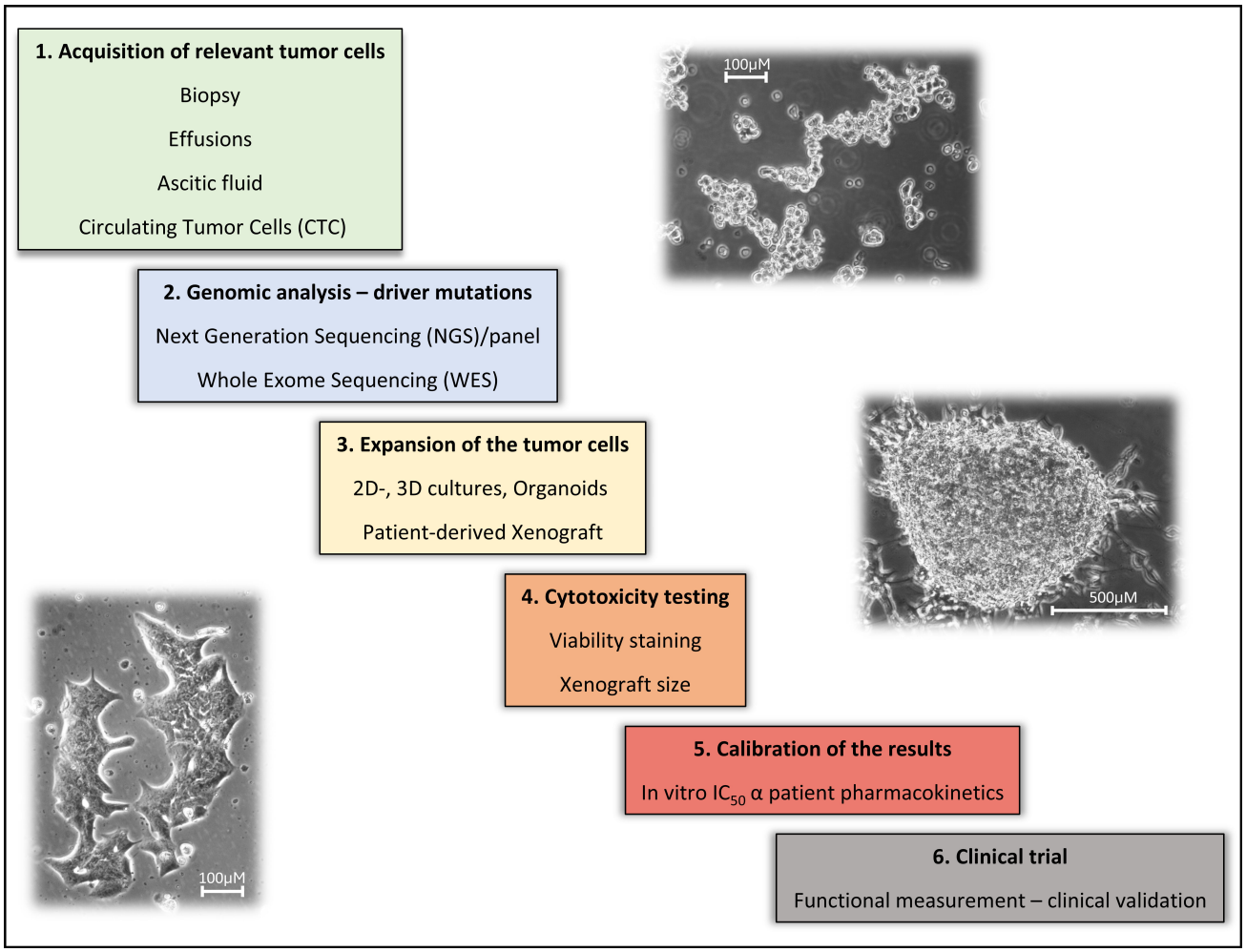

Fig. 1. Prerequisites of functional precision medicine. (1) Tumor cells are recovered by different modalities for (2) genomic analysis and determination of driver mutations. In most cases, chemosensitivity tests require a larger number of cells that need to be procured by (3) *in vitro* expansion prior to (4) viability assays. (5) The results of chemosensitivity determinations are then compared to drug concentrations that can be achieved in patients. Provided that all steps are successfully performed, the predictive value of the *ex vivo* measurements may be confirmed in prospective (6) clinical trials. Light microscopic pictures of cultivated tumor cells from pleural effusions show the different growth characteristics in the form of single cell suspensions, attached to the cell flask surface or as loosely attached spheroids. The figure was created using Microsoft PowerPoint.

NSCLC cells can be screened for lung cancer driver mutations that are not covered by hotspot NGS arrays used for pathological testing of mutated oncogenes. One example is the detection of a Son of Sevenless 1 (SOS1) driving mutation in a KRAS wild-type NSCLC cell line BH1406 [66].

Another application of FPM in NSCLC is the preclinical testing of drugs in an early phase of clinical development. FPM can aid in finding efficient drugs without the investigation of detailed mechanisms of resistance. A key problem for the interpretation of *in vitro* FPM tests is the drug concentration used and its relation to clinical pharmacokinetics. Studies involving NSCLC patients and pleura-derived cancer cells could be helpful to establish a relationship between IC_{50} values obtained *in vitro* and the clinical efficacy of the respective active drug. The use of pleura-derived tumor cells avoids problems encountered with organoid cultures in NSCLC that in most cases are overgrown by normal airway cells [67,68].

Preclinical Development of the KRAS G12D Inhibitor MRTX1133

The KRAS G12D inhibitor MRTX1133, the first of such inhibitors, was tested in a Phase I clinical trial [69]. This study of MRTX1133 in solid tumors harboring a KRAS G12D mutation (NCT05737706), enrolling 63 patients, was terminated prior to phase II in March 2025 due to high pharmacokinetic variability and failure to meet key activity thresholds. This failure is in contrast to the reported high activity of MRTX1133 in preclinical development employing KRAS G12D pancreatic cancer cell lines. In detail, using such lines, IC_{50} values below 5 nM were

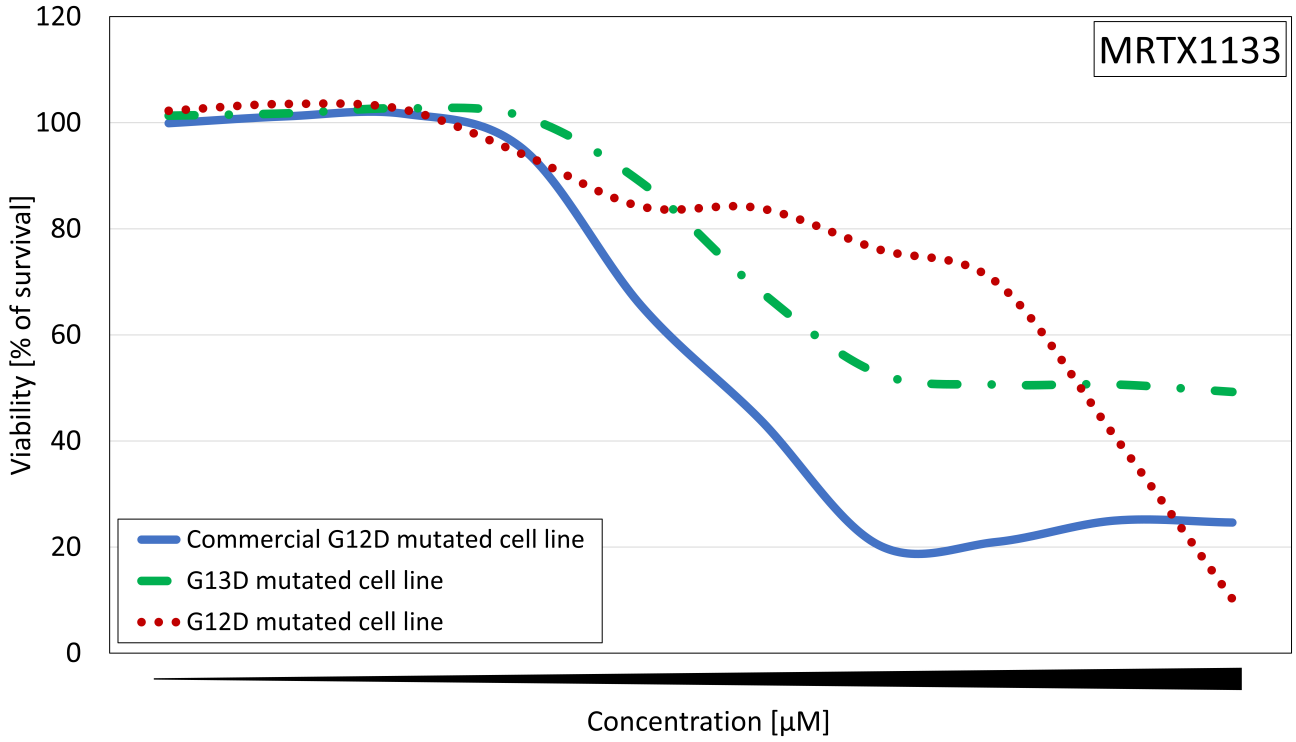

Fig. 2. Schematic representation of typical results of MRTX1133 cytotoxicity tests. Comparison of dose-response curves of a commercial G12D mutated cell line, a pleura-derived G13D mutated cell line, and a pleura-derived G12D mutated cell line (up to 10 µM of MRTX1133). The figure was created using Microsoft PowerPoint.

reported for MRTX1133 [70]. However, significant fractions of cells, reaching up to 30%, were still viable at high concentrations of up to 4 µM of MRTX1133 [71]. In an independent study checking pancreatic cell lines in 2D cultures, IC_{50} value sensitivities reported ranged from >100 to >5000 nM for MRTX1133 [72]. In combination with its relatively poor bioavailability, survival of cells at high concentrations, and possible development of chemoresistance, limitations in clinical efficacy were to be expected [73]. Typical schematic survival curves of cancer cell lines treated with MRTX1133 are shown in Fig. 2. While a commercially available permanent KRAS G12D line shows high chemosensitivity (ranging from 1 nM to 10 µM), a KRAS G13D line established from a pleural effusion showed chemoresistance and was outweighed by the resistance observed in a typical pleura-derived KRAS G12D NSCLC cell line. The failure of MRTX1133 in these cytotoxicity tests reveals the limitations of a single molecular epitope approach for attacking KRAS, with modest response rates, rapid development of resistance, restricted patient applicability, and reliance on highly sensitive permanent cell lines. Further developments may result in Proteolysis-targeting chimera (PROTAC)-based KRAS degraders that have the potential to overcome resistance seen with conventional inhibitors.

Sensitivity of Adenocarcinoma Cells to RMC-6236 Treatment

Revolution Medicine developed a series of KRAS glues to block the activity of all genotypes of KRAS by structurally hindering the activity of this oncogene via sanglifehrin-mediated attachment to Cyclophilin A [74,75]. RMC-6236 (Daraxonrasib) is active against all KRAS mutations, while other derivatives, such as RMC-9805, are optimized to target KRAS G12D [76]. By employing an allosteric approach, these RMC compounds circumvent the covalent binding problem entirely. For lung cancer and pancreatic cancer cell lines, IC_{50} values of single-digit nanomolar inhibitory concentrations were reported. Specifically, RMC-6236 activity was assessed across a panel of 845 cancer cell lines and was found to potently inhibit cell growth in KRAS G12X cells, with a median IC_{50} value of 8 nM [76]. For the NSCLC lines NCI-H23 and NCI-H358, IC_{50} values of 7.1 nM and 3.9 nM were determined.

Efficacy and safety results of a Phase I trial of RMC-6236 in patients with pancreatic ductal adenocarcinoma (PDAC) were recently reported at the 2025 Gastrointestinal Cancers Symposium [77]. For 127 patients with KRAS-mutant PDAC, the overall response rate (ORR, both confirmed and unconfirmed) was 27%, but for KRAS G12C, the ORR was 36%. For RMC-9805, a RAS(ON) G12D inhibitor, the ORR was 30% in 40 patients [78]. RMC-6236 showed a 38% overall response rate in RAS-mutant NSCLC

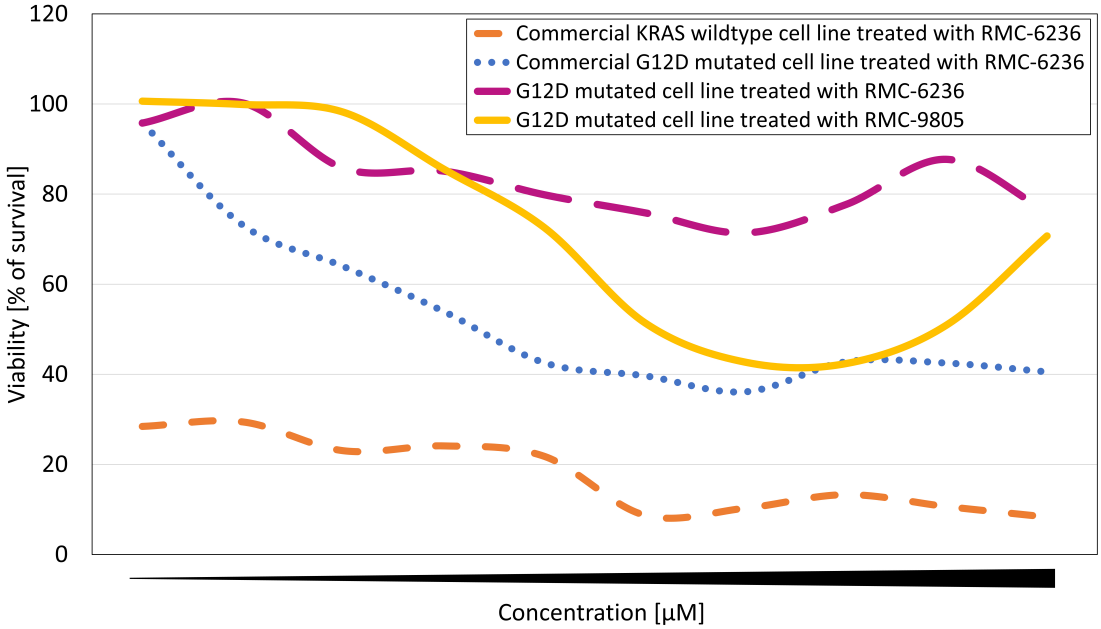

Fig. 3. Schematic representation of typical results of RMC-6236 and RMC-9805 cytotoxicity tests. Comparison of a commercial KRAS wildtype and G12D mutated cell line treated with RMC-6236, as well as a pleura-derived G12D cell line treated with RMC-6236 and RMC-9805 (up to 2 µM of the RMC compounds). The figure was created using Microsoft PowerPoint.

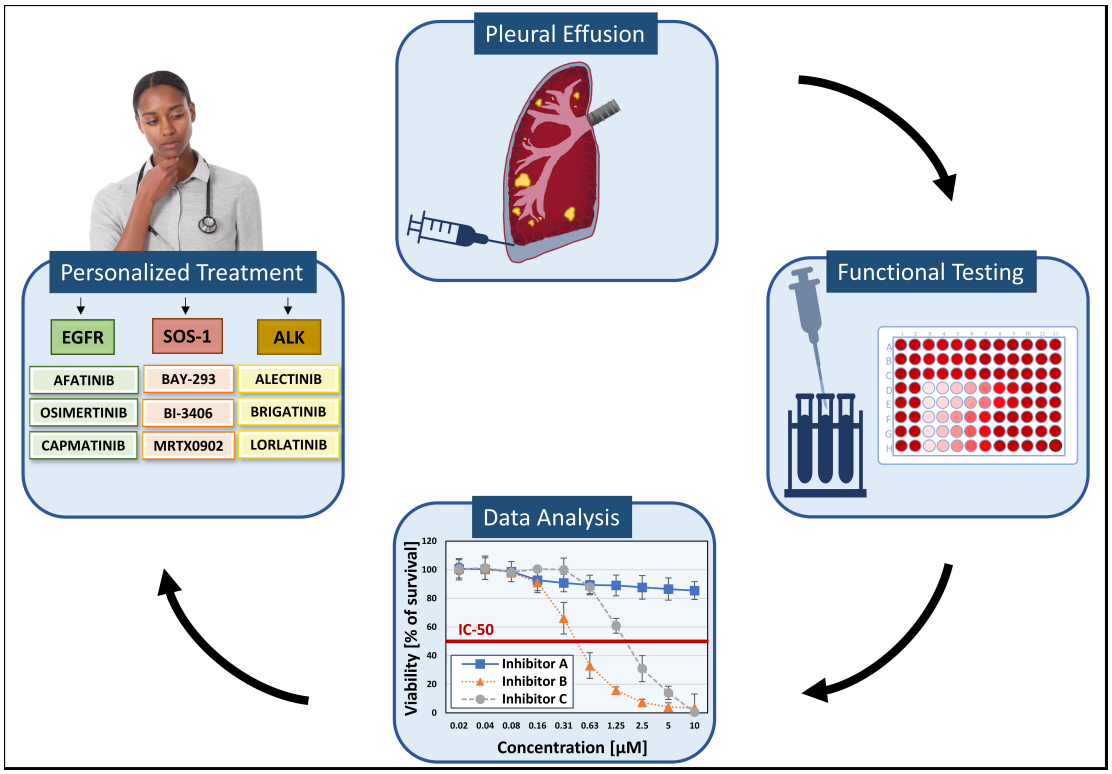

Fig. 4. Functional precision medicine in lung cancer. Lung cancer tumor cells can be obtained by draining pleural effusions. Isolated tumor cells are subjected to functional testing in short-term cytotoxicity testing in MTT stands for 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assays. The half maximal inhibitory concentration (IC_{50}) values of drugs under investigation are calculated from dose-response curves and compared to clinical peak plasma concentrations reported in the literature. Drugs, for which the *in vitro* IC_{50} concentrations exceed the clinically achievable peak plasma concentrations, are likely to fail in patients. The figure was created using Microsoft PowerPoint.

patients, with a median response duration of 15.1 months [79]. Median progression-free survival was 9.8 months, and median overall survival was 17.7 months for patients treated with RMC-6236. Treatment-related adverse effects led to dose modifications in 52% of patients, with rash as the most common adverse effect. However, the side effects on normal tissues were described as transient and manageable. Notably, RMC-6236 KRAS glue exhibits high activity in a commercially available KRAS wild-type cell line.

In an *in vitro* test, a typical commercial KRAS G12D line shows high chemosensitivity; however, approximately 40–50% of cells survived up to the highest concentration of the drug used in PDX models (so-called bottom fraction). In contrast, a real-world, pleura-derived lung cancer KRAS G12D cell line revealed high resistance to RMC-6236 up to 2 µM, while RMC-9805 was more effective, but still left about 50% of cells surviving high-dose treatment (Fig. 3). Therefore, the results of clinical trials are significantly lower than expected from the extremely low IC_{50} values reported for the permanent cell lines tested *in vitro*. Although this difference may be due to problems in *in vivo* bioavailability, the prominent surviving fraction of cells at high concentrations of the drug seems to contribute to the escape of tumor cells. In drug combinations, the RMC compounds are expected to sterically block KRAS degraders.

Conclusion

Nearly 75% of drugs under preclinical development eventually fail in clinical trials due to lack of efficacy or safety concerns. Cell lines used in the selection of promising drugs grow indefinitely but seem to have poor predictive power due to alterations upon prolonged *in vitro* cultivation. Multi-omics analyses are used to identify cell lines most similar to their originating tumor tissues; however, these data cannot fully predict the growth behavior or chemosensitivity of the cell lines. Freshly isolated cells may retain their original characteristics and can be obtained, for example, from pleura-derived tumor cells of lung cancer patients (Fig. 4). In contrast to permanent cell lines, freshly isolated cells exhibit higher resistance and sometimes show survival of a fraction of cells up to micromolar concentrations of the drug, indicating refractoriness to such drugs. Therefore, pleura-derived lung cancer cells and cell lines may be used to validate *in vitro* chemosensitivity and clinical response. Such cells may possess higher predictive value in the selection of drug candidates. Attempts to extend studies on pleura-derived tumor cells to cells from solid tumor tissue biopsies are in progress, but the acquisition of representative tumor cells from such tumor specimens is difficult. Isolation of tumor cells from effusions or ascites is less complex than from surgically resected solid tumors and offers a practical means to investigate drug responses in primary, patient-derived cells.

Availability of Data and Materials

Not applicable.

Author Contributions

GH conceived and supervised the review; co-designed the review methodology and inclusion criteria; contributed to development of the search strategy and interpretation of the evidence; drafted sections of the manuscript and critically revised it. MH and ME conducted data extraction; contributed to interpretation of the evidence; prepared and revised figures and critically reviewed the manuscript. SS drafted the original manuscript; co-designed the review methodology and inclusion criteria; developed and executed the search strategy; contributed to interpretation of the evidence and manuscript revision. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

Acknowledgment

The authors acknowledge the continuous endorsement by Dr. Theo Hohenheim.

Funding

This research received no external funding.

Conflict of Interest

The authors declare no conflict of interest.

References

[1] Dowden H, Munro J. Trends in clinical success rates and therapeutic focus. Nature Reviews. Drug Discovery. 2019; 18: 495–496. https://doi.org/10.1038/d41573-019-00074-z.

[2] Hay M, Thomas DW, Craighead JL, Economides C, Rosenthal J. Clinical development success rates for investigational drugs. Nature Biotechnology. 2014; 32: 40–51. https://doi.org/10.1038/nbt.2786.

[3] Van Norman GA. Limitations of Animal Studies for Predicting Toxicity in Clinical Trials: Part 2: Potential Alternatives to the Use of Animals in Preclinical Trials. JACC. Basic to Translational Science. 2020; 5: 387–397. https://doi.org/10.1016/j.jacbts.2020.03.010.

[4] Schuhmacher A, Hinder M, Brief E, Gassmann O, Hartl D. Benchmarking R&D success rates of leading pharmaceutical companies: an empirical analysis of FDA approvals (2006-2022). Drug Discovery Today. 2025; 30: 104291. https://doi.org/10.1016/j.drudis.2025.104291.

[5] Neve RM, Chin K, Fridlyand J, Yeh J, Baehner FL, Fevr T, *et al*. A collection of breast cancer cell lines for the study of function-

ally distinct cancer subtypes. Cancer Cell. 2006; 10: 515–527. https://doi.org/10.1016/j.ccr.2006.10.008.

[6] Drexler HG, Matsuo AY, MacLeod RA. Continuous hematopoietic cell lines as model systems for leukemia-lymphoma research. Leukemia Research. 2000; 24: 881–911. https://doi.org/10.1016/s0145-2126(00)00070-9.

[7] Geraghty RJ, Capes-Davis A, Davis JM, Downward J, Freshney RI, Knezevic I, *et al*. Guidelines for the use of cell lines in biomedical research. British Journal of Cancer. 2014; 111: 1021–1046. https://doi.org/10.1038/bjc.2014.166.

[8] Bose B, Bozdag S. Identifying cell lines across pan-cancer to be used in preclinical research as a proxy for patient tumor samples. Communications Biology. 2024; 7: 1101. https://doi.org/10.1038/s42003-024-06812-3.

[9] Gillet JP, Varma S, Gottesman MM. The clinical relevance of cancer cell lines. Journal of the National Cancer Institute. 2013; 105: 452–458. https://doi.org/10.1093/jnci/djt007.

[10] Tomczak K, Czerwińska P, Wiznerowicz M. The Cancer Genome Atlas (TCGA): an immeasurable source of knowledge. Contemporary Oncology (Poznan, Poland). 2015; 19: A68–A77. https://doi.org/10.5114/wo.2014.47136.

[11] Roschke AV, Tonon G, Gehlhaus KS, McTyre N, Bussey KJ, Lababidi S, *et al*. Karyotypic complexity of the NCI-60 drug-screening panel. Cancer Research. 2003; 63: 8634–8647.

[12] Daniel VC, Marchionni L, Hierman JS, Rhodes JT, Devereux WL, Rudin CM, *et al*. A primary xenograft model of small-cell lung cancer reveals irreversible changes in gene expression imposed by culture in vitro. Cancer Research. 2009; 69: 3364–3373. https://doi.org/10.1158/0008-5472.CAN-08-4210.

[13] Tveit KM, Pihl A. Do cell lines in vitro reflect the properties of the tumours of origin? A study of lines derived from human melanoma xenografts. British Journal of Cancer. 1981; 44: 775–786. https://doi.org/10.1038/bjc.1981.276.

[14] Barretina J, Caponigro G, Stransky N, Venkatesan K, Margolin AA, Kim S, *et al*. The Cancer Cell Line Encyclopedia enables predictive modelling of anticancer drug sensitivity. Nature. 2012; 483: 603–607. https://doi.org/10.1038/nature11003.

[15] Yang W, Soares J, Greninger P, Edelman EJ, Lightfoot H, Forbes S, *et al*. Genomics of Drug Sensitivity in Cancer (GDSC): a resource for therapeutic biomarker discovery in cancer cells. Nucleic Acids Research. 2013; 41: D955–D961. https://doi.org/10.1093/nar/gks1111.

[16] Mirabelli P, Coppola L, Salvatore M. Cancer Cell Lines Are Useful Model Systems for Medical Research. Cancers. 2019; 11: 1098. https://doi.org/10.3390/cancers11081098.

[17] Liu K, Newbury PA, Glicksberg BS, Zeng WZD, Paithankar S, Andrechek ER, *et al*. Evaluating cell lines as models for metastatic breast cancer through integrative analysis of genomic data. Nature Communications. 2019; 10: 2138. https://doi.org/10.1038/s41467-019-10148-6.

[18] Warren A, Chen Y, Jones A, Shibue T, Hahn WC, Boehm JS, *et al*. Global computational alignment of tumor and cell line transcriptional profiles. Nature Communications. 2021; 12: 22. https://doi.org/10.1038/s41467-020-20294-x.

[19] Cancer Genome Atlas Research Network. Comprehensive genomic characterization defines human glioblastoma genes and core pathways. Nature. 2008; 455: 1061–1068. https://doi.org/10.1038/nature07385.

[20] Rodríguez MR, Biedma BA, Rodríguez Pérez I, Romeo JA. Elucidating the Role of *KRAS*, *NRAS*, and *BRAF* Mutations and Microsatellite Instability in Colorectal Cancer via Next-Generation Sequencing. Cancers. 2025; 17: 2071. https://doi.org/10.3390/cancers17132071.

[21] Hao W, Rajendran BK, Cui T, Sun J, Zhao Y, Palaniyandi T, *et al*. Advances in predicting breast cancer driver mutations: Tools for precision oncology (Review). International Journal of Molecular Medicine. 2025; 55: 6. https://doi.org/10.3892/ijmm.2024.5447.

[22] Monks A, Scudiero D, Skehan P, Shoemaker R, Paull K, Vistica D, *et al*. Feasibility of a high-flux anticancer drug screen using a diverse panel of cultured human tumor cell lines. Journal of the National Cancer Institute. 1991; 83: 757–766. https://doi.org/10.1093/jnci/83.11.757.

[23] Domcke S, Sinha R, Levine DA, Sander C, Schultz N. Evaluating cell lines as tumour models by comparison of genomic profiles. Nature Communications. 2013; 4: 2126. https://doi.org/10.1038/ncomms3126.

[24] Hamacher R, Bauer S. Preclinical models for translational sarcoma research. Current Opinion in Oncology. 2017; 29: 275–285. https://doi.org/10.1097/CCO.0000000000000373.

[25] Foglizzo V, Cocco E, Marchiò S. Advanced Cellular Models for Preclinical Drug Testing: From 2D Cultures to Organ-on-a-Chip Technology. Cancers. 2022; 14: 3692. https://doi.org/10.3390/cancers14153692.

[26] Sun D, Gao W, Hu H, Zhou S. Why 90% of clinical drug development fails and how to improve it? Acta Pharmaceutica Sinica. B. 2022; 12: 3049–3062. https://doi.org/10.1016/j.apsb.2022.02.002.

[27] Ibarrola-Villava M, Cervantes A, Bardelli A. Preclinical models for precision oncology. Biochimica et Biophysica Acta. Reviews on Cancer. 2018; 1870: 239–246. https://doi.org/10.1016/j.bbcan.2018.06.004.

[28] Hylander BL, Punt N, Tang H, Hillman J, Vaughan M, Bshara W, *et al*. Origin of the vasculature supporting growth of primary patient tumor xenografts. Journal of Translational Medicine. 2013; 11: 110. https://doi.org/10.1186/1479-5876-11-110.

[29] Miserocchi G, Mercatali L, Liverani C, De Vita A, Spadazzi C, Pieri F, *et al*. Management and potentialities of primary cancer cultures in preclinical and translational studies. Journal of Translational Medicine. 2017; 15: 229. https://doi.org/10.1186/s12967-017-1328-z.

[30] Janik K, Popeda M, Peciak J, Rosiak K, Smolarz M, Treda C, *et al*. Efficient and simple approach to in vitro culture of primary epithelial cancer cells. Bioscience Reports. 2016; 36: e00423. https://doi.org/10.1042/BSR20160208.

[31] Killekar K, Puranik SI, Akbar AA, Ghagane SB, Nerli RB, Hiremath M. Overview of primary cell culture models in preclinical research of prostate and bladder cancer. In Zhan X (ed.) Cell Culture-Advanced Technology and Applications in Medical and Life Sciences. IntechOpen: London, UK. 2022. https://doi.org/10.5772/intechopen.99493.

[32] Dangles-Marie V, Pocard M, Richon S, Weiswald LB, Assayag F, Saulnier P, *et al*. Establishment of human colon cancer cell lines from fresh tumors versus xenografts: comparison of success rate and cell line features. Cancer Research. 2007; 67: 398–407. https://doi.org/10.1158/0008-5472.CAN-06-0594.

[33] Shen C, Ferro EG, Xu H, Kramer DB, Patell R, Kazi DS. Underperformance of Contemporary Phase III Oncology Trials and Strategies for Improvement. Journal of the National Comprehensive Cancer Network: JNCCN. 2021; 19: 1072–1078. https://doi.org/10.6004/jnccn.2020.7690.

[34] Hamilton G, Rath B, Plangger A, Hochmair M. Implementation of functional precision medicine for anaplastic lymphoma kinase-rearranged non-small lung cancer. Precision Cancer Medicine. 2019; 2: 19.

[35] Szulkin A, Szatmári T, Hjerpe A, Dobra K. Chemosensitivity and resistance testing in malignant effusions with focus on primary malignant mesothelioma and metastatic adenocarcinoma. Pleura and Peritoneum. 2016; 1: 119–133. https://doi.org/10.1515/pp-2016-0013.

[36] Jovanovic D. Etiopathogenesis of malignant pleural effusion. AME Medical Journal. 2021; 6: 28. https://doi.org/10.21037/am

j-2019-mpe-05.
[37] Meditz K, Rinner B. Establishment of tumor cell lines: From primary tumor cells to a tumor cell line. In Kasper C, Charwat V, Lavrentieva A, (eds.) Cell Culture Technology. Learning Materials in Biosciences (pp. 61–73). Springer: Cham. 2018. https://doi.org/10.1007/978-3-319-74854-2_4.
[38] Kodack DP, Farago AF, Dastur A, Held MA, Dardaei L, Friboulet L, *et al*. Primary Patient-Derived Cancer Cells and Their Potential for Personalized Cancer Patient Care. Cell Reports. 2017; 21: 3298–3309. https://doi.org/10.1016/j.celrep.2017.11.051.
[39] Kim SY, Lee JY, Kim DH, Joo HS, Yun MR, Jung D, *et al*. Patient-Derived Cells to Guide Targeted Therapy for Advanced Lung Adenocarcinoma. Scientific Reports. 2019; 9: 19909. https://doi.org/10.1038/s41598-019-56356-4.
[40] Gazdar AF, Gao B, Minna JD. Lung cancer cell lines: Useless artifacts or invaluable tools for medical science? Lung Cancer (Amsterdam, Netherlands). 2010; 68: 309–318. https://doi.org/10.1016/j.lungcan.2009.12.005.
[41] Yablonskii P, Nefedov A, Arseniev A, Kozak A, Mortada M, Patsyuk A. Non-small cell lung cancer, pleural effusion and carcinomatosis: always a criterion of inoperability? AME Medical Journal. 2020; 5: 10. https://doi.org/10.21037/amj.2020.02.07.
[42] Allemani C, Matsuda T, Di Carlo V, Harewood R, Matz M, Nikšić M, *et al*. Global surveillance of trends in cancer survival 2000-14 (CONCORD-3): analysis of individual records for 37 513 025 patients diagnosed with one of 18 cancers from 322 population-based registries in 71 countries. Lancet (London, England). 2018; 391: 1023–1075. https://doi.org/10.1016/S0140-6736(17)33326-3.
[43] Jonna S, Subramaniam DS. Molecular diagnostics and targeted therapies in non-small cell lung cancer (NSCLC): an update. Discovery Medicine. 2019; 27: 167–170.
[44] Schwartzberg L, Kim ES, Liu D, Schrag D. Precision Oncology: Who, How, What, When, and When Not? American Society of Clinical Oncology Educational Book. Annual Meeting. 2017; 37: 160–169. https://doi.org/10.1200/EDBK_174176.
[45] Casolino R, Beer PA, Chakravarty D, Davis MB, Malapelle U, Mazzarella L, *et al*. Interpreting and integrating genomic tests results in clinical cancer care: Overview and practical guidance. CA: A Cancer Journal for Clinicians. 2024; 74: 264–285. https://doi.org/10.3322/caac.21825.
[46] Lim TKH, Skoulidis F, Kerr KM, Ahn MJ, Kapp JR, Soares FA, *et al*. KRAS G12C in advanced NSCLC: Prevalence, co-mutations, and testing. Lung Cancer (Amsterdam, Netherlands). 2023; 184: 107293. https://doi.org/10.1016/j.lungcan.2023.107293.
[47] Romero D. Two new agents target KRAS G12C. Nature Reviews. Clinical Oncology. 2020; 17: 6. https://doi.org/10.1038/s41571-019-0304-3.
[48] Lanman BA, Allen JR, Allen JG, Amegadzie AK, Ashton KS, Booker SK, *et al*. Discovery of a Covalent Inhibitor of $KRAS^{G12C}$ (AMG 510) for the Treatment of Solid Tumors. Journal of Medicinal Chemistry. 2020; 63: 52–65. https://doi.org/10.1021/acs.jmedchem.9b01180.
[49] Wu LL, Jiang WM, Liu ZY, Zhang YY, Qian JY, Liu Y, *et al*. AMG-510 and cisplatin combination increases antitumor effect in lung adenocarcinoma with mutation of KRAS G12C: a preclinical and translational research. Discover Oncology. 2023; 14: 91. https://doi.org/10.1007/s12672-023-00698-z.
[50] Fell JB, Fischer JP, Baer BR, Blake JF, Bouhana K, Briere DM, *et al*. Identification of the Clinical Development Candidate MRTX849, a Covalent $KRAS^{G12C}$ Inhibitor for the Treatment of Cancer. Journal of Medicinal Chemistry. 2020; 63: 6679–6693. https://doi.org/10.1021/acs.jmedchem.9b02052.
[51] Luo W, Zhu J, Zhang W, Yu A, Zhou W, Xu K. Efficacy and toxicity of drugs targeting $KRAS^{G12C}$ mutation in non-small cell lung cancer: a meta-analysis. Expert Review of Anticancer Therapy. 2023; 23: 1295–1303. https://doi.org/10.1080/14737140.2023.2282606.
[52] Hofmann MH, Gmachl M, Ramharter J, Savarese F, Gerlach D, Marszalek JR, *et al*. BI-3406, a Potent and Selective SOS1-KRAS Interaction Inhibitor, Is Effective in KRAS-Driven Cancers through Combined MEK Inhibition. Cancer Discovery. 2021; 11: 142–157. https://doi.org/10.1158/2159-8290.CD-20-0142.
[53] Hofmarcher T, Malmberg C, Lindgren P. A global analysis of the value of precision medicine in oncology - The case of non-small cell lung cancer. Frontiers in Medicine. 2023; 10: 1119506. https://doi.org/10.3389/fmed.2023.1119506.
[54] Yang SR, Schultheis AM, Yu H, Mandelker D, Ladanyi M, Büttner R. Precision medicine in non-small cell lung cancer: Current applications and future directions. Seminars in Cancer Biology. 2022; 84: 184–198. https://doi.org/10.1016/j.semcancer.2020.07.009.
[55] Flobak Å, Skånland SS, Hovig E, Taskén K, Russnes HG. Functional precision cancer medicine: drug sensitivity screening enabled by cell culture models. Trends in Pharmacological Sciences. 2022; 43: 973–985. https://doi.org/10.1016/j.tips.2022.08.009.
[56] Salmon SE, Hamburger AW, Soehnlen B, Durie BG, Alberts DS, Moon TE. Quantitation of differential sensitivity of human-tumor stem cells to anticancer drugs. The New England Journal of Medicine. 1978; 298: 1321–1327. https://doi.org/10.1056/NEJM197806152982401.
[57] Shoemaker RH, Wolpert-DeFilippes MK, Kern DH, Lieber MM, Makuch RW, Melnick NR, *et al*. Application of a human tumor colony-forming assay to new drug screening. Cancer Research. 1985; 45: 2145–2153.
[58] Selby P, Buick RN, Tannock I. A critical appraisal of the "human tumor stem-cell assay". The New England Journal of Medicine. 1983; 308: 129–134. https://doi.org/10.1056/NEJM198301203080304.
[59] Von Hoff DD. "Send this patient's tumor for culture and sensitivity". The New England Journal of Medicine. 1983; 308: 154–155. https://doi.org/10.1056/NEJM198301203080310.
[60] Malani D, Kumar A, Brück O, Kontro M, Yadav B, Hellesøy M, *et al*. Implementing a Functional Precision Medicine Tumor Board for Acute Myeloid Leukemia. Cancer Discovery. 2022; 12: 388–401. https://doi.org/10.1158/2159-8290.CD-21-0410.
[61] Kornauth C, Pemovska T, Vladimer GI, Bayer G, Bergmann M, Eder S, *et al*. Functional Precision Medicine Provides Clinical Benefit in Advanced Aggressive Hematologic Cancers and Identifies Exceptional Responders. Cancer Discovery. 2022; 12: 372–387. https://doi.org/10.1158/2159-8290.CD-21-0538.
[62] Popova AA, Levkin PA. Precision medicine in oncology: in vitro drug sensitivity and resistance test (DSRT) for selection of personalized anticancer therapy. Advanced Therapeutics. 2020; 3: 1900100. https://doi.org/10.1002/adtp.201900100.
[63] Manzari MT, Shamay Y, Kiguchi H, Rosen N, Scaltriti M, Heller DA. Targeted drug delivery strategies for precision medicines. Nature Reviews. Materials. 2021; 6: 351–370. https://doi.org/10.1038/s41578-020-00269-6.
[64] Letai A, Bhola P, Welm AL. Functional precision oncology: Testing tumors with drugs to identify vulnerabilities and novel combinations. Cancer Cell. 2022; 40: 26–35. https://doi.org/10.1016/j.ccell.2021.12.004.
[65] van Renterghem AWJ, van de Haar J, Voest EE. Functional precision oncology using patient-derived assays: bridging genotype and phenotype. Nature Reviews. Clinical Oncology. 2023; 20: 305–317. https://doi.org/10.1038/s41571-023-00745-2.
[66] Hamilton G, Stickler S, Ermakov M, Eggerstorfer MT, Nocera

FP, Hohenegger M, *et al*. Characterization of the BH1406 non-small cell lung cancer (NSCLC) cell line carrying an activating SOS1 mutation. Translational Lung Cancer Research. 2024; 13: 2987–2997. https://doi.org/10.21037/tlcr-24-570.

[67] Dijkstra KK, Monkhorst K, Schipper LJ, Hartemink KJ, Smit EF, Kaing S, *et al*. Challenges in Establishing Pure Lung Cancer Organoids Limit Their Utility for Personalized Medicine. Cell Reports. 2020; 31: 107588. https://doi.org/10.1016/j.celrep.2020.107588.

[68] Gao B, Huang C, Kernstine K, Pelekanou V, Kluger Y, Jiang T, *et al*. Non-malignant respiratory epithelial cells preferentially proliferate from resected non-small cell lung cancer specimens cultured under conditionally reprogrammed conditions. Oncotarget. 2017; 8: 11114–11126. https://doi.org/10.18632/oncotarget.14366.

[69] Wang X, Allen S, Blake JF, Bowcut V, Briere DM, Calinisan A, *et al*. Identification of MRTX1133, a Noncovalent, Potent, and Selective $KRAS^{G12D}$ Inhibitor. Journal of Medicinal Chemistry. 2022; 65: 3123–3133. https://doi.org/10.1021/acs.jmedchem.1c01688.

[70] Christensen JG. Discovery and characterization of MRTX1133, a selective non-covalent inhibitor of KRASG12D. In AACR-NCI-EORTC Virtual International Conference on Molecular Targets and Cancer Therapeutics. San Diego, California. American Association for Cancer Research. 2021.

[71] Wei D, Wang L, Zuo X, Maitra A, Bresalier RS. A Small Molecule with Big Impact: MRTX1133 Targets the KRASG12D Mutation in Pancreatic Cancer. Clinical Cancer Research: an Official Journal of the American Association for Cancer Research. 2024; 30: 655–662. https://doi.org/10.1158/1078-0432.CCR-23-2098.

[72] Tajiknia V, Pinho-Schwermann M, Srinivasan PR, Hernandez Borrero L, Zhang L, Huntington KE, *et al*. Synergistic antitumor activity, reduced pERK, and immuno-stimulatory cytokine profiles with 5-FU or ONC212 plus KRAS G12D inhibitor MRTX1133 in CRC and pancreatic cancer cells independent of G12D mutation. American Journal of Cancer Research. 2024; 14: 4523–4536. https://doi.org/10.62347/DVXL1377.

[73] Stickler S, Rath B, Hamilton G. Targeting KRAS in pancreatic cancer. Oncology Research. 2024; 32: 799–805. https://doi.org/10.32604/or.2024.045356.

[74] Holderfield M, Lee BJ, Jiang J, Tomlinson A, Seamon KJ, Mira A, *et al*. Concurrent inhibition of oncogenic and wild-type RAS-GTP for cancer therapy. Nature. 2024; 629: 919–926. https://doi.org/10.1038/s41586-024-07205-6.

[75] Jiang J, Jiang L, Maldonato BJ, Wang Y, Holderfield M, Aronchik I, *et al*. Translational and Therapeutic Evaluation of RAS-GTP Inhibition by RMC-6236 in RAS-Driven Cancers. Cancer Discovery. 2024; 14: 994–1017. https://doi.org/10.1158/2159-8290.CD-24-0027.

[76] Jiang L, Menard M, Weller C, Wang Z, Burnett L, Aronchik I, *et al*. RMC-9805, a first-in-class, mutant-selective, covalent and oral KRASG12D(ON) inhibitor that induces apoptosis and drives tumor regression in preclinical models of KRASG12D cancers. Cancer Research. 2023; 83: 526. https://doi.org/10.1158/1538-7445.AM2023-526.

[77] Garrido-Laguna I, Wolpin BM, Park W, Azad NS, Spira AI, Starodub A, *et al*. Safety, efficacy, and on-treatment circulating tumor DNA (ctDNA) changes from a phase 1 study of RMC-6236, a RAS (ON) multi-selective, tri-complex inhibitor, in patients with RAS mutant pancreatic ductal adenocarcinoma (PDAC). Journal of Clinical Oncology. 2025; 43. https://doi.org/10.1200/JCO.2025.43.4_suppl.722.

[78] Revolution Medicines. Revolution Medicines Presents Initial Data from RMC-9805 Monotherapy Study in Patients with Advanced Pancreatic Ductal Adenocarcinoma. 2024. Available at: https://ir.revmed.com/news-releases/news-release-details/revolution-medicines-presents-initial-data-rmc-9805-monotherapy (Accessed: 26 June 2025).

[79] Punekar S, Hong DS, Luo J, Ou SI, Johnson ML, Spira A, *et al*. Safety and clinical activity of daraxonrasib (RMC-6236) in RAS mutant non–small cell lung cancer (NSCLC). Journal of Thoracic Oncology. 2025; 20: S10–S11. https://doi.org/10.1016/S1556-0864(25)00201-1.