Genialis

Genialis™ krasID

PLATFORM ALGORITHMS FOR MECHANISTIC INSIGHTS AND DRUG RESPONSE PREDICTION

WHITE PAPER

CONTENT:

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1. Introduction: Questions that matter

RAS mutations are among the most common oncogenic drivers in human cancer, occurring in more than a quarter of all cases worldwide. The approval of KRAS inhibitors such as sotorasib and adagrasib has ushered in a new era of targeted therapy. Yet the promise of these drugs has been tempered by reality: fewer than half of patients benefit, and for most that do, responses are modest or short-lived.

For translational researchers and clinical developers, this raises urgent and practical questions:

- Which patients will actually benefit from my KRAS inhibitor, and why?
- How long will their response last?
- Can we predict *a priori* mechanisms that confer potential innate or adaptive resistance?
- Why do some patients progress early despite the mutation being "targetable"?
- Can we identify rational combinations to extend the benefit?
- Can we design trials that are faster, leaner, and more likely to succeed?

Traditional DNA-based biomarkers cannot provide these answers. KRAS mutation profiling is standard-of-care, but it only indicates whether a patient's tumor harbors a KRAS variant. It does not capture whether that tumor is truly dependent on KRAS signaling, nor does it explain why many mutation-positive patients fail to respond. Even among responders, KRAS mutation status offers no insight into durability or resistance. More elaborate studies of co-mutation spectra hint at resistance mechanisms, but have yet to prove predictive in any clinical setting.

RNA, by contrast, provides a snapshot of the tumor's active biology. It reflects not just mutational potential but the actual cellular states, pathway activity, and microenvironmental context that determine whether a KRAS inhibitor will work. When paired with explainable AI, RNA biomarkers transform raw transcriptomic data into clinically meaningful insights that address the questions researchers and clinicians most urgently need answered.

Genialis™ krasID was built precisely for this purpose. By modeling core KRAS biology and its bypass mechanisms, krasID moves beyond mutation status to predict treatment response, forecast duration of benefit, and illuminate resistance biology. **krasID is a modular, explainable, and customizable platform that derives mechanistic insight, guides rational trial design, and supports clinical decision-making.**

This whitepaper presents the science behind krasID and highlights case studies across preclinical and clinical settings. It shows how this platform can help drug developers leverage a greater understanding of the tumor's biology into better clinical outcomes.

krasID is a modular, explainable, and customizable platform that derives mechanistic insight, guides rational trial design, and supports clinical decision-making.

2. A platform for compound-specific KRASi phenomarkers

For more than a decade, precision oncology has relied primarily on DNA mutation testing to guide treatment. In the KRAS field, the emergence of G12C inhibitors transformed this genetic information into therapeutic action: if the mutation is present, prescribe the drug. But as clinical experience has shown, the presence of a mutation is not sufficient for response, and response is often not durable. What is missing is a biomarker that captures not just the potential for a drug to bind its target, but also the actual tumor biology that governs response and resistance. Gene expression profiling offers such a solution by measuring pathway activity, microenvironmental forces, and the tumor's dependency on KRAS signaling.

The solution is a new class of biomarkers, **RNA phenomarkers**, that approximate biological states and phenotypes rather than just genetic variants. Genialis krasID is the first algorithm platform designed specifically to develop such phenomarker algorithms for KRAS inhibitors (KRASi) in order to derive mechanistic insights into KRASi biology or predict response to KRASi in preclinical models and patients.

The solution is a new class of biomarkers, RNA phenomarkers, that approximate biological states and phenotypes rather than just genetic variants.

2.1 krasID delivers RNA phenomarkers

RNA is emerging as the key clinical analyte powering a new class of biomarkers that serve as highly accurate patient classifiers. Historically, RNA has been seen as less clinically tractable due to assay costs, sample variability, and data reproducibility issues. These concerns have been addressed through advancements in RNA sequencing technology and analytical processing. RNA sequencing has become highly reliable, affordable, and commoditized, making its use in clinical applications more attractive.

Standardized methods for sample preservation, nucleic acid extraction, and sequencing preparation contribute to high reproducibility across labs, even with small archival samples. The availability of sequencing services means RNA-seq is integrated into clinical workflows and can be reliably extracted and quantified from FFPE slides without needing fresh biopsies or frozen tissue. The ability to multiplex different tests from the same analyte and decreasing sequencing costs make transcriptomic biomarkers cost-effective for complex disease diagnosis and clinical R&D. Advances like FoundationOne®RNA and Tempus XR demonstrate the technical and commercial feasibility of this approach in clinical diagnostics.

The era of RNA phenomarkers has arrived.

Traditional DNA biomarkers	Genialis RNA biomarkers
DNA represents **potential** biological states, or what a cell could do	RNA represents **actual** biological states, or what a cell does
One to a few DNA variants	Hundreds-to-thousands of genes accounted for by quantitative signatures comprising their expression and variants
Narrow scope: one status, one gene	Broad scope: Complex multi-modal and multi-function analysis
Binary Q+A: *Is the mutation present?*	Complex Q+A: *Will the underlying biology of the tumor respond to therapy?*
Property of the drug target *Is there a target that the drug can bind to?* *Are you eligible for a certain drug?*	Biology of a patient tumor *Will this drug actually work?* *Is it meaningful to a particular tumor?*
Able to identify patients that **may receive drug**	Able to identify patients that **should receive drug** *AND* forecast the response to that drug
NO information on treatment duration or combination strategies	Stratifies patients based on treatment duration and survival; Provides actionable strategies for combination treatment

◀ TABLE 1

The case for RNA phenomarkers.

2.2 krasID overcomes challenges of predictive AI in emerging therapies

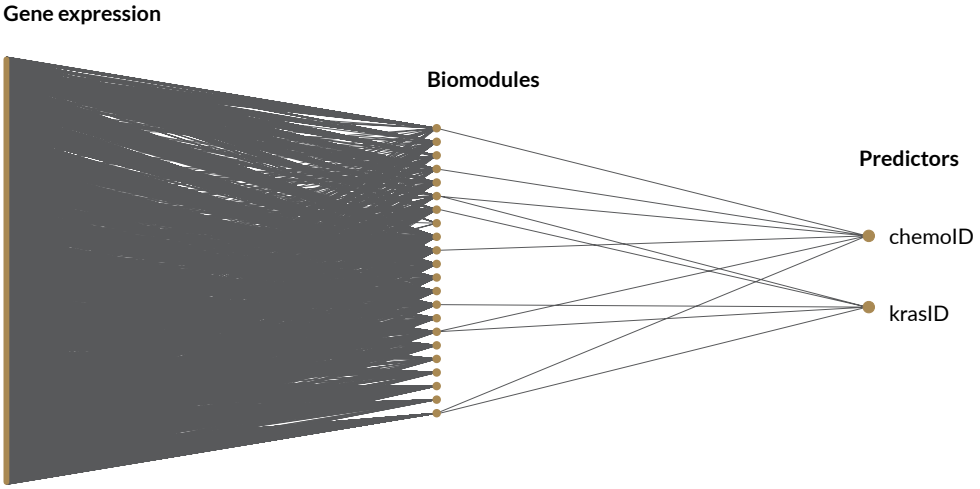
Artificial intelligence (AI) stands to transform precision medicine by using large 'omics datasets to develop evermore complete and precise biomarkers powered by sophisticated machine learning (ML) algorithms. However, several major obstacles remain to introducing AI-based tools into clinical decision making:

1. Most AI tools lack explainability of the selected features (so-called black-box methods), and are therefore less attractive to regulators and physicians.
2. Most clinical 'omics datasets contain vastly more measurements (e.g., genes) and orders of magnitude fewer patients, which leads to overfitting and reduced reproducibility.

3. Definitionally, novel (investigational) therapeutics have not been administered to many patients; thus, the available clinical data is limited and often lacks response endpoints. Therefore, one cannot readily train an algorithm to learn treatment outcome from a population sample of meaningful size.

Together, these challenges impede the development of AI-powered, widely-used, reproducible, and clinically accepted predictors. **Genialis krasID is different.**

Unlike black-box methods, Genialis krasID combines human expertise with an explainable AI architecture to capture the complexity of KRAS biology and reduce it to clinical utility. krasID is built on the Genialis™ Supermodel framework, a large molecular model (LMM) that transforms raw RNA-seq data into interpretable biomodules. Often AI models themselves, biomodules are algorithmic representations of diverse biological mechanisms, pathways, and relationships, each representing a specific aspect of biology and using different input genes. Some biomodules directly relate to KRAS (e.g., dependency, MAPK activation), while others involve external factors with indirect implications (e.g., immune system, tumor microenvironment, hormone signaling, etc). The Genialis Supermodel comprises a comprehensive library of cancer biomodules, from which a select few may be combined into a phenomarker algorithm for any specific prediction task. Computational data-driven methods are used in concert with "expert" (manual) filters derived from our understanding of cancer biology for this feature selection. Compared to using gene expression values directly for prediction, the biomodule feature set is dramatically reduced in dimensionality and carries relevant and interpretable biological signal, making it possible to derive accurate models (predictors) from small experimental or clinical cohorts.

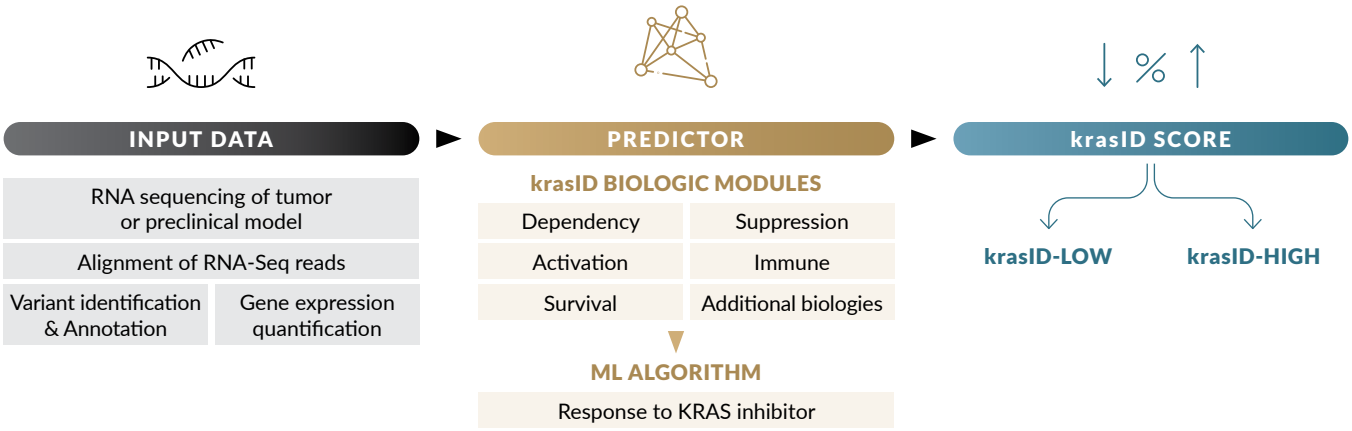


◀ **FIGURE 1**

Dimensionality reduction is achieved by combining hundreds of gene expression values into a handful of biomodule scores which are then used to train new predictors or make predictor calls. Note: similar to krasID, chemoID is a platform for developing predictors for chemotherapy.

A single predictor is thus an ensemble model that integrates a subset of these biomodules to generate a robust phenomarker score, which is the probability of a particular outcome to KRAS inhibition (*Figure 1*). Different kinds of predictors can be trained, for example, for predicting the probability of response to therapy or the

duration of response. The exact composition of a krasID predictor can be fine-tuned for particular compounds, mutation profiles, and disease histologies. The resulting predictor scores follow a bimodal distribution that ensures a clear decision boundary between krasID-High vs. krasID-Low, regardless of the specific numerical threshold. Additionally, krasID provides per-sample scores for each biomodule, offering deeper insights into each patient's tumor landscape.



▲ FIGURE 2

Genialis krasID integrates multiple biomodules to create predictors, e.g., for predicting response to KRAS inhibition. Left - Gene expression data is measured by RNA sequencing of tumor tissue (including FFPE samples). Raw RNA-Seq data are processed to extract normalized gene expression profiles and identify genomic variants. Middle - Preprocessed data serves as input for a number of KRAS-related or KRAS-adjacent biomodules. Biomodule output is then used as input for an AI-based predictor. Right - The output of the predictor is a score, e.g., the probability of response to KRASi therapy.

2.3 Biomodules yield customizable and interpretable predictors

krasID is built on the Genialis Supermodel framework. It is a large molecular model that transforms raw RNA-seq data into interpretable biomodule scores. Biomodules model the central tenets of cancer biology, such as those described as Hallmarks of Cancer[1]. These Hallmarks include such biologies as resisting cell death, sustaining proliferative signaling, and evading immune surveillance. Modules range in complexity from measuring pathway signaling activity to inferring the immune status of the tumor microenvironment. Genialis trains and validates these biomodules using a repository of hundreds of thousands of public and proprietary datasets spanning various model systems, including preclinical models (e.g., cell lines, xenografts) and real-world tumor biopsies collected from ethno-geographically diverse populations. This approach ensures each module is informative, unbiased, and transferable across multiple potential use cases.

A subset of these modules can be used individually for surveying specific aspects of tumor biology. Alternatively, a careful selection of these modules, e.g., based on the mechanism of action of a drug, can be used as input features for modeling

The modular design of krasID means it is not a single biomarker, but a platform for building custom predictors to serve as phenomarkers.

1 Hanahan D., Hallmarks of Cancer: New Dimensions. Cancer Discov. 2022 Jan;12(1):31-46. doi: 10.1158/2159-8290.CD-21-1059. PMID: 35022204.

predictors, e.g., the response to a specific therapeutic target. This flexible approach assigns specific combinations of modules to particular data types, drug mechanisms, or intended uses (*Table 2*).

The modular design of krasID means it is not a single biomarker, but a platform for building custom predictors to serve as phenomarkers. Different KRAS inhibitors exploit different mechanisms, and krasID can be tailored to each. Picking from a couple of dozen carefully selected and validated biomodules, new predictors can be rapidly configured and fine-tuned to maximize predictive accuracy for specific drug development programs.

Unlike black-box AI, krasID is inherently explainable. Each biomodule corresponds to a tangible mechanism, pathway, or phenotype. When predictors are built on top of these biomodules, predictions are transparent and biologically grounded. This foundation sets the stage for the next chapter, where we show how krasID performs in practice across preclinical studies and real-world patient cohorts.

▼ TABLE 2

A selection of krasID biomodules used to model predictors highlighted in Chapter 3 - Case studies

Biomodule	Biomodule Description	Biomodule Construction	Biomodules used for response modeling: Preclinical (G12C)	Preclinical (PanRAS)	Clinical
KRAS dependency	Infers the dependency of cancer cells on KRAS for survival and proliferation.	A 10 gene feature set selected using a linear regression model with recursive feature elimination and ElasticNet regularization. Validation used repeated nested cross-validation.	✓	✓	✓
MAPK/ERK activity	Measures the amount of MAPK signaling in cancer cells.	~500 gene feature set selected using a weighted linear regression model incorporating robust and stably expressed gene sets. Validation used leave-one-out cross-validation.	✓	✓	✓
PI3K activity	Measures the amount of PI3K signaling in cancer cells.	~500 gene feature set selected using a weighted linear regression model incorporating robust and stably expressed gene sets. Validation used leave-one-out cross-validation.	-	✓	✓
TP53 activity	Measures the amount of TP53 activity in cancer cells.	~500 gene feature set selected using a weighted linear regression model incorporating robust and stably expressed gene sets. Validation used leave-one-out cross-validation.	-	-	✓
Immune activity	Provides an assessment of the tumor microenvironment immune status.	A 13 gene feature set comprising stably annotated genes was used to compute an immune activation score using a linear model.	-	-	✓
Other biologies	20+ modules capturing aspects of intrinsic (e.g., apoptosis, hormone signaling), and extrinsic KRAS biology (e.g., angiogenesis, TGF-beta signaling)	"Explainable" machine learning leveraging linear, logistic, and foundation-modeling approaches trained on Genialis™ Expressions library of >1M harmonized whole transcriptomic records	-	-	✓

3. Case studies

This chapter provides three examples of krasID in action, predictors trained for different drugs and experimental setups.

1. **Preclinical Proof-of-Concept:** A 2-biomodule predictor accurately predicting in vitro and in vivo responses to KRAS G12C inhibitors (sotorasib, adagrasib, etc.) in lung cancer models.
2. **Pan-Cancer Analysis:** Trained using 3 core biomodules, this predictor stratified responsive vs. resistant cell lines across NSCLC, PDAC, and CRC for a novel pan-KRAS inhibitor.
3. **Real-World Patient Study:** In a cohort of 66 real-world KRAS G12C-selected NSCLC patients treated with sotorasib, a 5-module krasID model predicted not only which patients responded, but also for how long. Predictor calls can be clinically interpreted.

3.1 Preclinical proof of concept

The two biomodules used in this predictor are KRAS dependency and MAPK pathway activation. A logistic regression classifier to predict low- or high-IC50 values was trained on published IC50 values measured from various sources. We hypothesized that preclinical models reliant on KRAS-mediated oncogenic signaling for proliferation and survival are those most susceptible to KRAS inhibition.

▼ TABLE 3

Performance of a predictor trained on two krasID biomodules (dependency and activation) predicting durable KRAS inhibitor response in three types of preclinical models. Short-term (e.g., IC50) and sustained cytotoxicity (e.g., PRISM Repurposing dataset) metrics were classified into 'low' and 'high' categories via K-means clustering for each model system. A durable response was defined when a model system exhibited both short-term and prolonged cytotoxicity.

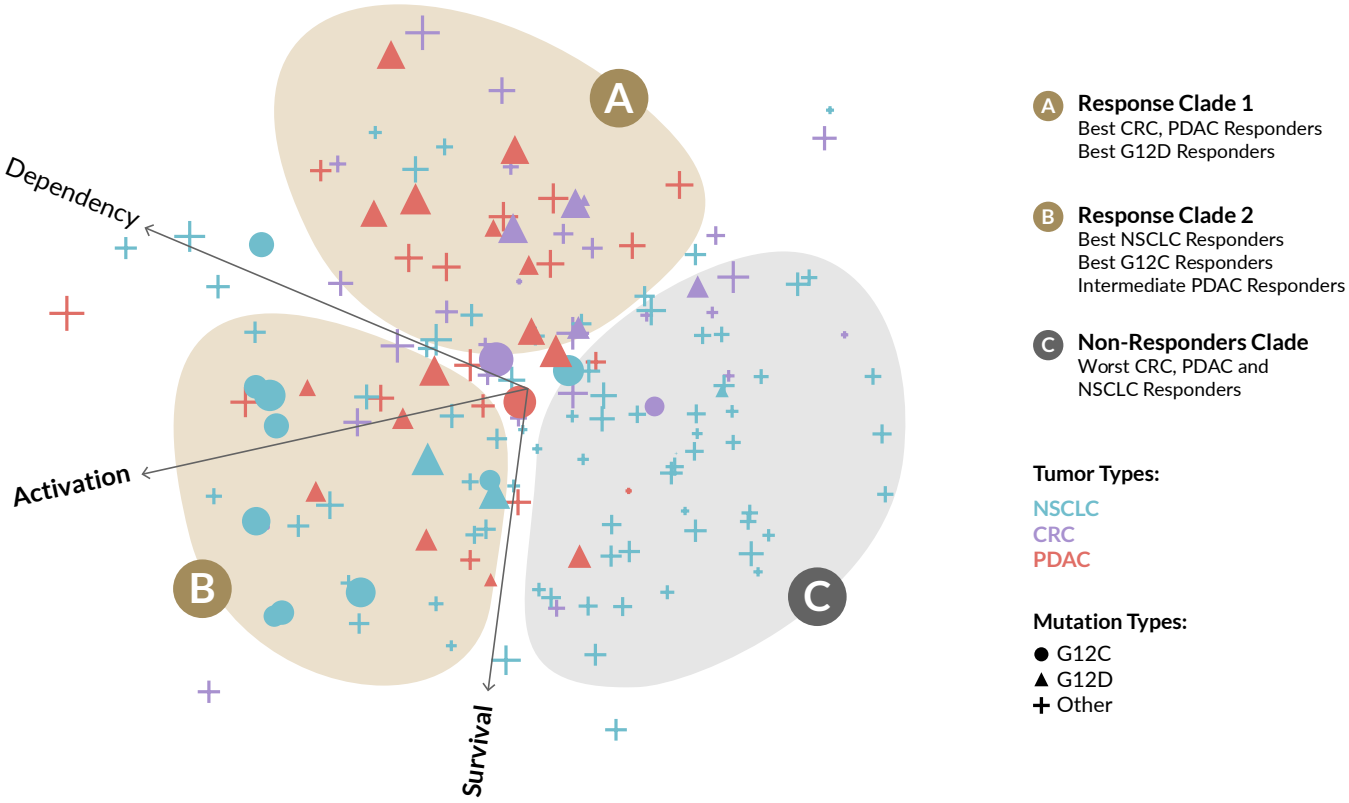
Drug Name	Company	Mechanism of Action	Preclinical Model System	N*	Sensitivity	Specificity	AUROC
Sotorasib	Amgen	G12C-OFF	2D	11	1.0 (5 / 5)	1.0 (6 / 6)	1
RMC-6291	Revolution Medicine	G12C-ON	2D	15	1.0 (7 / 7)	0.88 (7 / 8)	0.96
Adagrasib	Bristol Myers Squibb	G12C-OFF	2D	12	1.0 (6 / 6)	0.67 (4 / 6)	0.94
Adagrasib	Bristol Myers Squibb	G12C-OFF	3D	12	0.88 (7 / 8)	0.75 (3 / 4)	0.81
Adagrasib	Bristol Myers Squibb	G12C-OFF	Xenograft	10	1.0 (5 / 5)	0.8 (4 / 5)	0.96
All drugs (Genialis krasID)	-	-	All	67	0.97 (33 / 34)	0.85 (28 / 33)	0.94
All drugs (Dummy Model)	-	-	All	67	0.53 (18 / 34)	0.64 (21 / 33)	0.59

*Displayed performance metrics on preclinical model systems that have more than 10 data points, as well as on the entire dataset.
2D: 2-dimensional cell culture | **3D:** 3-dimensional cell culture | **N:** Available models | **AUROC:** Area Under the Receiver Operating Curve

To test this hypothesis, we evaluated publicly available cytotoxicity responses to KRAS G12C-inhibitors (Amgen's sotorasib, Bristol Myers Squibb's adagrasib, and Revolution Medicine's RMC-6291) across various preclinical non-small cell lung cancer (NSCLC) model systems, including 2D/3D cell cultures and xenografts. On average, the predictor accurately stratified cell line responders, achieving a receiver operating characteristic (AUROC) of 0.94 compared to 0.59 for a dummy "null" model (*Table 3*).

3.2 Pan-cancer analysis

Identifying responders to various G12C inhibitors using intrinsic biological modules suggested the applicability of krasID across different histologies and mutation settings. To test this, we tailored a model on cytotoxicity values measured in 161 NSCLC, PDAC, and CRC cell lines treated with the RevMed PanRAS inhibitor RMC7977. Responses were evaluated using principal component analysis (PCA) with three krasID modules: KRAS dependency, Activation (MAPK pathway activity), and Survival (PI3K pathway activity). From a projection of these three modules, distinct



▲ FIGURE 3

PCA showing cytotoxicity responses to RAS(ON) multi-selective inhibitor in NSCLC, PDAC, and CRC cell lines. Responses are stratified using three krasID modules. Datapoint size corresponds to RMC7977 sensitivity.[2]

2 Cytotoxicity values were obtained from Holderfield et al., 2024.

clusters representing responders or non-responders to panRAS inhibition emerged based on mutational and histological profiles (*Figure 3*). This demonstrated that the multimodular architecture of krasID can account for tissue- or mutation-specific tumor biologies, highlighting the potential to customize response biomarkers across different histologies, mutational subtypes, and drug targets.

3.3 Real-world patient study

This section details the utility of krasID in evaluating real-world patient responses to Amgen's KRAS G12C inhibitor, sotorasib (Lumakras). The results highlight three key points:

1. **Predicting patient response to KRASi:** A krasID predictor trained using 5 biomodules optimized for G12C NSCLC human subjects accurately predicted clinical response in a real-world dataset (ROC AUC = 0.81), demonstrating its utility as a clinically informative phenomarker.
2. **Predicting duration of response:** A krasID predictor trained using 17 biomodules accurately classified patients into short- and long-term responders (<6 and >6 months, respectively) (AUC ROC = 0.80).
3. **Interpreting krasID predictions:** Biomodule scores offered potentially clinically actionable insights into sotorasib response and progression in a longitudinal cohort (pre- and post-treatment samples).

This study analyzed a real-world cohort of 66 patients with KRAS G12C-mutated NSCLC who had received at least 4 weeks of sotorasib treatment. The real-world sotorasib-treated cohort closely mirrored the demographics of the multicenter, single-group, open-label sotorasib clinical trial, CodeBreak 100[3], suggesting that results may be extrapolated to the clinical trial cohort. Longitudinal data were available for five patients with biopsies at the time of relapse. Given constraints on the metadata associated with real-world datasets, this study used time-on-treatment (ToT) as a surrogate for progression-free survival, and defined clinical benefit as having achieved a complete response (CR), partial response (PR), or stable disease (SD) as the best overall response.

Together, these findings illustrate that the utility of krasID is not limited to baseline patient selection. Rather, it functions as a dynamic, biology-informed guide to managing KRAS inhibitor therapy across the treatment journey. For patients, this means more personalized care and the possibility of extended responses. For clinical teams and developers, it means actionable hypotheses to optimize treatment strategy and design next-generation trials.

3 Skoulidis F., et al., Sotorasib for Lung Cancers with KRAS p.G12C Mutation. N Engl J Med. 2021 Jun 24;384(25):2371-2381. doi: 10.1056/NEJMoa2103695. PMID: 34096690 and https://clinicaltrials.gov/study/NCT03600883

3.3.1 Predicting patient response to KRASi

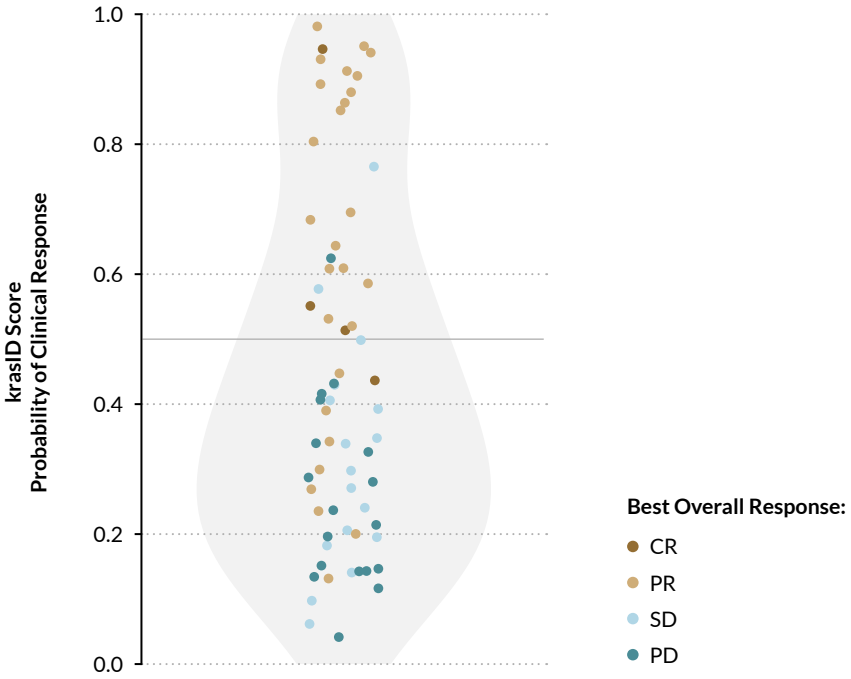
We evaluated the performance of a number of algorithm architectures and module combinations in predicting response to the aforementioned real-world sotorasib dataset. We hypothesized that the increased complexity and heterogeneity of in vivo tumor biology would require more sophisticated module composition, as compared to the preclinical use case.

▼ TABLE 4

The top-performing model was a regression algorithm that incorporated five krasID biological modules, including the dependency and activation modules, to infer clinical response. It accurately predicted responders in ~80% of the cases, an improvement compared to the measured response rate (49%) with the standard-of-care KRAS biomarker, which relies on mutational assessment alone. Dummy model metrics are provided to benchmark the classifier's performance, assuming random predictions aligned with the dataset's class distribution. The performance of the dummy model is expected to be at the same level as predicting response based on genotype alone (i.e., a coin flip).

	Accuracy	Precision	Recall	Specificity	F1 Score	ROC AUC
Clinical Response	0.79	0.84	0.68	0.89	0.75	0.81
Baseline (Dummy)	0.55	0.51	0.58	0.51	0.55	0.55

Accuracy: Number of correct predictions / Total number of predictions
Precision: Positive Predictive Value (PPV) = True biomarker responses / Total predicted biomarker response
Recall: Sensitivity = True biomarker responses / Total actual responses
Specificity: True Negative Rate = True biomarker non-responses / Total actual non-responses
F1 Score: Harmonic mean of precision and recall
AUROC: Area Under the Receiver Operating Curve



▲ FIGURE 4

Probability of clinical response for a sotorasib-treated NSCLC cohort. Individual data points represent a single patient colored by clinical outcome.

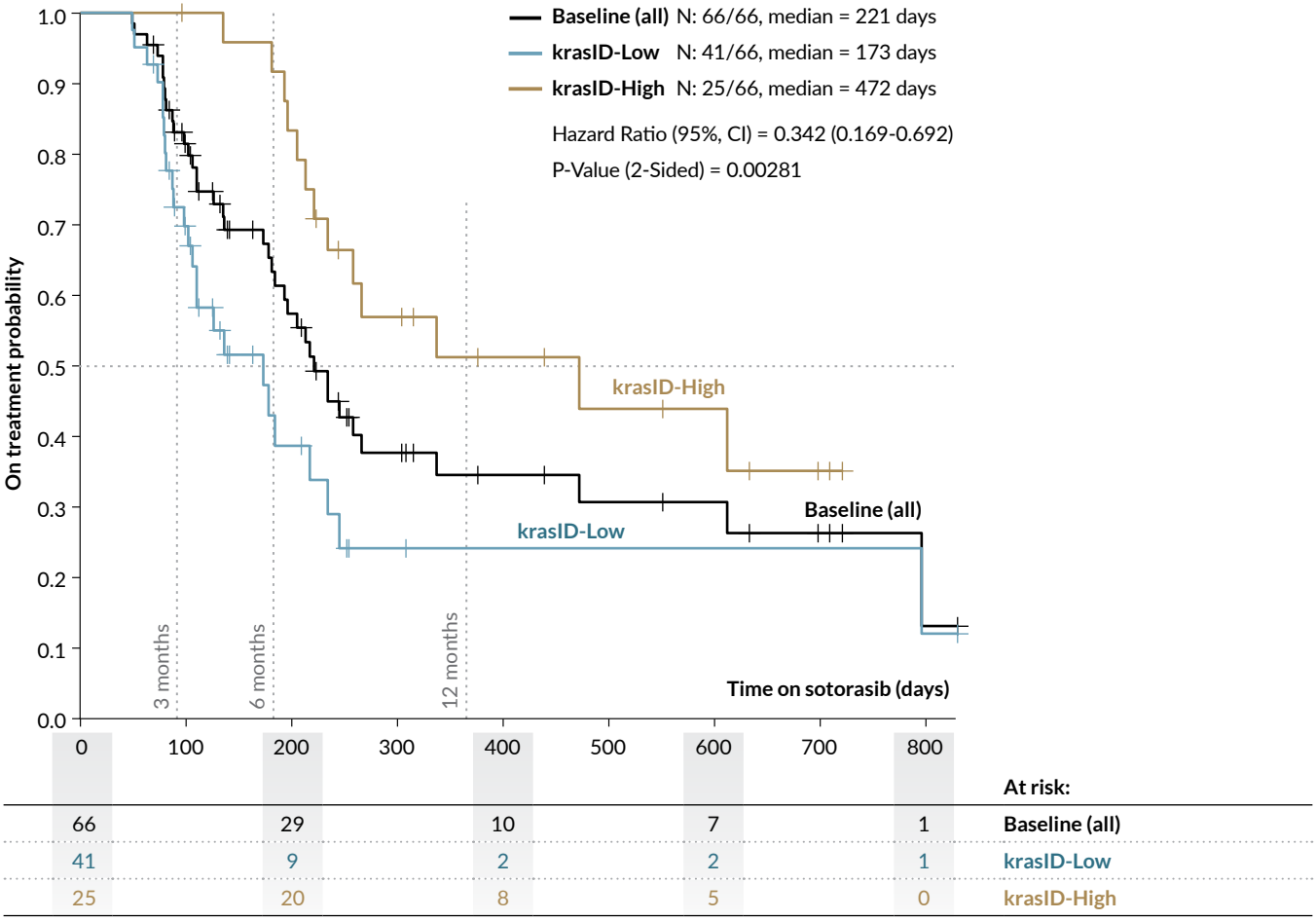
To train and fine-tune the patient classifier, a total of 20 distinct biomodules were created to reflect both KRAS intrinsic and extrinsic tumor biologies. Modules served as features for logistic regression with an L2 penalty to predict clinical response (CR + PR). Regularization was optimized using 10-fold, 3x repeated stratified cross-validation (CV) with log loss as the metric. The model was then evaluated using leave-one-out CV. The best-performing model's performance metrics are provided in *Table 4*. The classifier outputs a continuous probability of response that can be cleanly thresholded, allowing for a simple biomarker designation of krasID-HIGH or LOW for each patient (*Figure 4*). Thresholds may be established empirically to optimize sensitivity, specificity, or overall accuracy, or can be set to achieve pre-specified rates of inclusion/exclusion.

Among the 66 patients, an objective response (CR or PR) occurred in 31 patients (47%), while clinical benefit (disease control indicated by a CR, PR, or SD) was observed in 48 patients (73%). A Kaplan-Meier estimate of time on treatment using Genialis krasID to predict patient response revealed significant differences in median treatment durations: 472 days for those with predicted clinical response (krasID-High) compared to 173 days for those with no predicted clinical benefit (krasID-Low) (Hazard Ratio = 0.342, p-value = 0.002). The median treatment duration for all patients, selected by mutation status but not stratified by krasID, was 221 days (*Table 5; Figure 5*).

▼ TABLE 5

Comparison of sotorasib clinical activity between CodeBreak100 clinical trial and real-world cohort with krasID-Low and krasID-High subsets.

	Codebreak100	Present Study	krasID-Low	krasID-High
ORR - %	37.10%	47.00%	22.00%	88.00%
Best Overall Response - no. (%)	124	66	41	25
CR	4 (3.2%)	4 (6.1%)	1 (2.4%)	3 (12.0%)
PR	42 (33.9%)	27 (40.9%)	8 (19.5%)	19 (76.0%)
SD	54 (43.5%)	17 (25.8%)	15 (36.6%)	2 (8.0%)
PD	20 (16.1%)	18 (27.3%)	17 (41.5%)	1 (4.0%)
Not evaluable	4 (3.2%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Time on treatment (ToT) median (range) - days	167 (6 - 542)	221 (49 - 830)	173 (49 - 830)	472 (96 - 721)
Progression free survival median - days	207			
PFS or ToT* after 3 months	~70%	83%	72%	100%
PFS or ToT* after 6 months	52%	63%	43%	92%
PFS or ToT* after 12 months	~30%	35%	24%	51%



▲ FIGURE 5

Kaplan-Meier survival curves for time on treatment as stratified by Genialis krasID

In conclusion, krasID predictor stratified KRAS-mutated patients into responders and nonresponders showing not only statistically but also clinically meaningful improvement of patient selection when compared to the standard of care biomarker.

3.3.2 Predicting duration of response

Examining the relationship between krasID status and time on treatment in this sotorasib in the previous use case revealed that krasID status may provide insights into the duration of response. Specifically, among krasID-High patients, 92% remained responsive at 6 months (compared to 72.8% of those with confirmed response in CodeBreaK200[4]), and 51% remained responsive at 12 months (compared to 50.6% of those with confirmed response in CodeBreaK200).

4 de Langen AJ et al., Sotorasib versus docetaxel for previously treated non-small-cell lung cancer with KRASG12C mutation: a randomised, open-label, phase 3 trial. Lancet. 2023 Mar 4;401(10378):733-746. doi: 10.1016/S0140-6736(23)00221-0. PMID: 36764316. and https://clinicaltrials.gov/study/NCT04303780

To test if krasID could predict durability, we tuned another predictor using 17 biomodules (see also section 3.3.3. or details) that stratified patients into "<6 months" vs. ">6 months" time-on-treatment groups. It achieved AUROC = 0.80, significantly outperforming random baseline models and mutation-only selection strategies (*Table 6*). Patients predicted to remain on therapy >180 days had a median time on treatment of 337 days, compared to 126 days for those predicted to progress earlier. The stratification was statistically significant ($p = 6.15 \times 10^{-5}$).

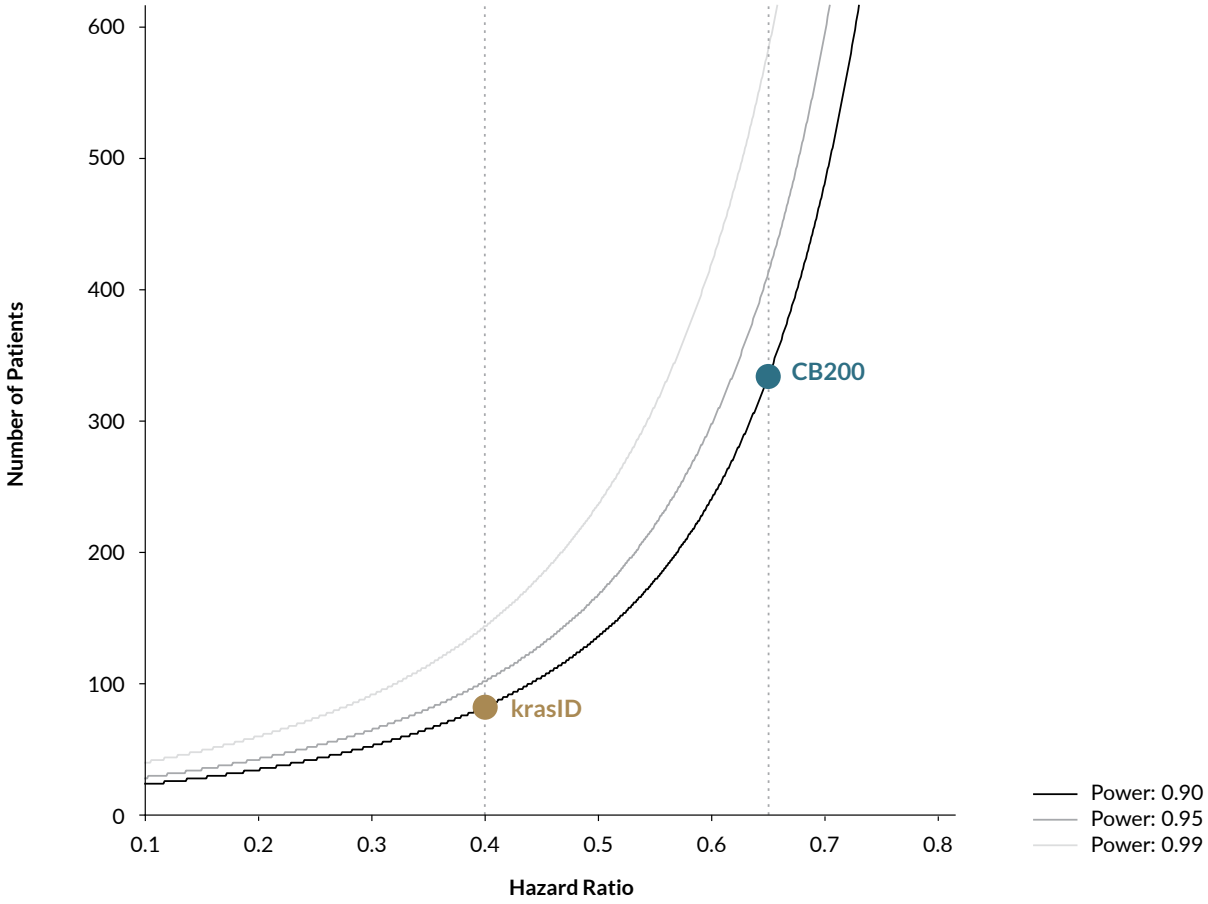
▼ TABLE 6

Predictor performance for duration of response to sotorasib. The 180-day cutoff was chosen based on the 5.6-month median PFS in CodeBreaK 200, reflecting a clinically relevant milestone for progression. Individual biological module scores served as input features for a classification model. Tuning and model refinement were performed with nested leave-one-out cross-validation, resulting in a model that achieved an AUROC of 0.80.

	Accuracy	Precision	Recall	Specificity	F1 Score	ROC AUC
ToT classifier at 180 days	0.78	0.78	0.69	0.85	0.73	0.80
Dummy model	0.51	0.44	0.42	0.58	0.43	0.48

Accuracy: Number of correct predictions / Total number of predictions
Precision: Positive Predictive Value (PPV) = True biomarker responses / Total predicted biomarker response
Recall: Sensitivity = True biomarker responses / Total actual responses
Specificity: True Negative Rate = True biomarker non-responses / Total actual non-responses
F1 Score: Harmonic mean of precision and recall
AUROC: Area Under the Receiver Operating Curve
ToT: Time on Treatment
Dummy control models are used by predicting the most common class ('common class strategy') or guessing randomly based on the class frequencies '(stratified class strategy'). Shown is a stratified class strategy that best reflects the real class distribution.

This case study highlights a unique value of Genialis krasID in clinical development. By going beyond simple mutation status, the model demonstrated the ability to forecast the durability of response. This capability is critical for drug developers who wish to use biomarkers to enrich trials for durable responders. For example, if a krasID sotorasib biomarker were available for use during phase 2 or beyond, it could power a similarly sized trial with significantly lower enrollment. Achieving 90% power at a 0.05 significance level for the CodeBreak 200 trial required 333 patients (krasG12C mutation alone selected at an HR of 0.65), compared to 82 patients with krasID stratification at a postulated hazard ratio of 0.40. This results in an approximately four-fold reduction in the number of patients required for enrollment (*Figure 6*). Although the FDA is unlikely to approve a Phase 3 trial design with fewer than 100 patients, such biomarker stratification could either provide increased power (>99%) or lead to accelerated approval based on an early interim analysis of krasID biomarker-stratified patients. In monetary terms, conservative estimates ($80k per patient, $1M operational) posit $25M for CB200 compared to ~$7.6M for a krasID-stratified trial, representing a 3.3-fold cost reduction.



▲ FIGURE 6

Required sample size for the CB200 trial and a trial using krasID (lower hazard ratio). Customizing krasID to a G12C inhibitor results in a 4-fold reduction in the total number of patients required to power a non-biomarker stratified trial, translating to an estimated 3-fold cost reduction.

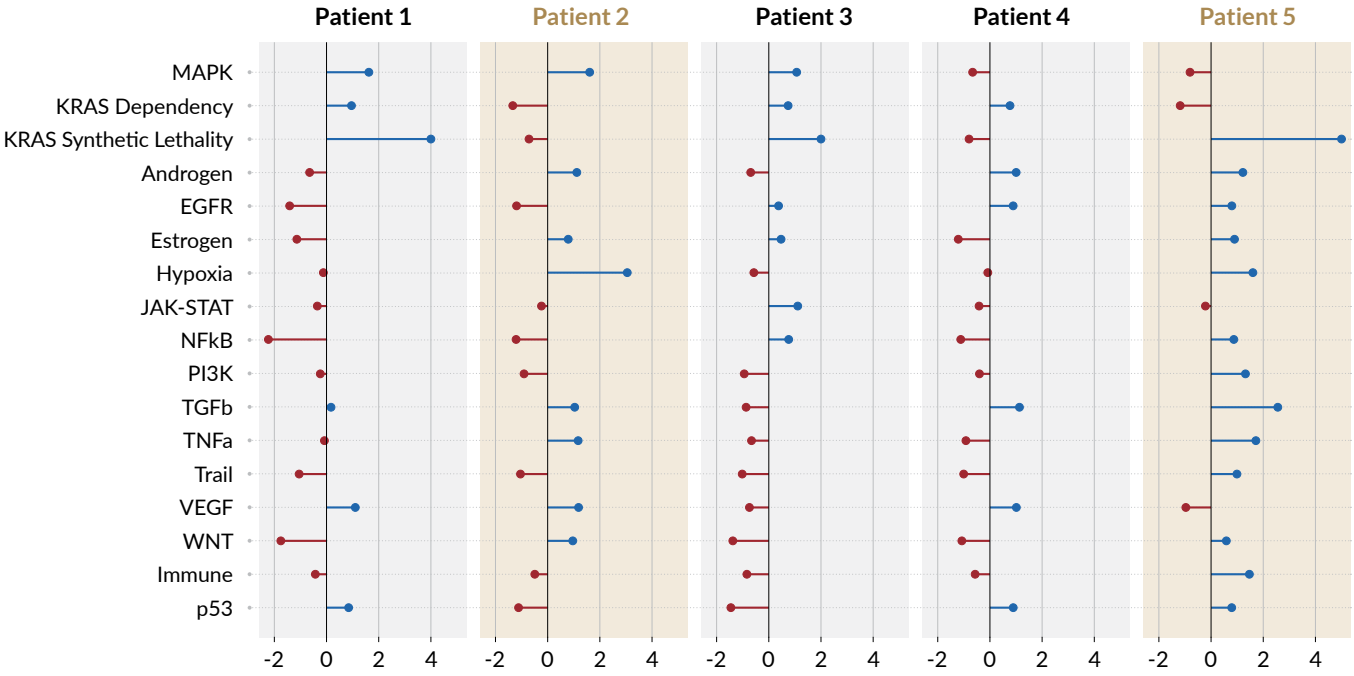
3.3.3 Interpreting krasID predictions

While predicting which patients will respond to KRAS inhibitors and for how long is a core function of krasID, helping understand why patients eventually progress is equally important. Resistance biology defines not only the clinical durability of KRAS inhibitors but also the rationale for next-line or combination therapies. By profiling patients both before and after treatment, the krasID predictor sheds light on biological shifts that occur during therapy, uncovering mechanisms of resistance that are invisible to DNA mutation testing. This is critical for clinicians, who must set expectations and plan treatment sequencing.

A subset of five patients in the real-world sotorasib cohort provided both pretreatment and post-progression biopsies. These longitudinal samples were analyzed with krasID, focusing on fold changes in biomodule activity scores between the two time points (*Figure 7*). This approach enabled identification of both shared and patient-specific resistance mechanisms, while preserving visibility into KRAS-driven biology.

We highlight two case examples:

- **Patient 2** did not respond to sotorasib. Post-treatment profiling revealed increased MAPK activity and reduced KRAS dependency, suggesting ineffective target modulation by sotorasib. Elevated TGFβ, VEGF, and Hypoxia modules indicate a shift toward extrinsic microenvironmental resistance. It may be advisable to discontinue sotorasib therapy. The patient may benefit from targeting bypassed stromal/angiogenic pathways (e.g., TGFβ, VEGF, HIF-2 inhibitors).
- **Patient 5** first achieved a partial response (PR) to sotorasib, then progressed. Post-treatment samples showed decreased MAPK and KRAS dependency scores consistent with on-target drug activity. However, increased PI3K, WNT/β-catenin, and Immune module scores suggested adaptive resistance via bypass signaling. Patient may still be benefiting from sotorasib, and discontinuation risks MAPK rebound. A combination approach targeting upregulated bypass pathways while maintaining KRAS inhibition may be warranted to prolong clinical benefit.



▲ FIGURE 7

krasID biomodule score changes may translate into potentially actionable decisions.

4. Getting started with Genialis krasID

Genialis krasID is not a single phenomarker algorithm; it is a predictor platform. While Genialis can certainly run existing predictors on new data, your clinical development program will likely benefit from a tailored implementation incorporating your experimental data and a compound-specific selection of biology-informed features (biomodules). This yields a predictor tuned to your drug, experimental system, and disease indication. This chapter explains how we configure a new predictor in collaboration with our partners, and how those predictors become usable phenomarkers to guide drug development and patient care.

4.1 Training a new predictor

The process of creating a new krasID predictor follows a structured, iterative workflow designed to ensure interpretability, reproducibility, and clinical utility.

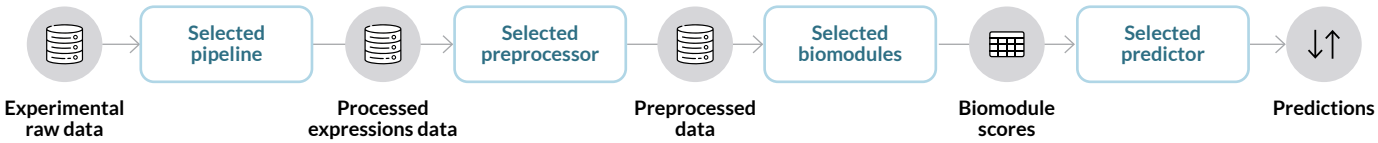
1. **Biomarker scoping.** Every predictor begins with a clear problem statement: which biological or clinical question should the model answer? For example, forecasting duration of response to a KRAS inhibitor, stratifying responders vs. non-responders, or identifying resistance mechanisms. Available outcome data (clinical benefit, IC_{50} values, survival times) are curated into balanced training sets, while potential confounders and outliers are carefully managed.
2. **Feature Selection.** Gene expression data are transformed into biomodule scores by the Genialis Supermodel. Over two dozen biomodules, each capturing a specific aspect of KRAS-related or -adjacent biology, are evaluated. Feature selection combines biology-guided curation (modules related to drug MoA) with data-driven ranking (regularization, recursive elimination). Poorly reproducible or non-transferable modules are removed.
3. **Model Training.** Several machine learning approaches are tested, from interpretable linear models (e.g., logistic regression, ElasticNet) to non-linear methods (tree ensembles, neural networks). Models are evaluated for accuracy, stability, convergence, and explainability.
4. **Internal Validation.** Performance is estimated using nested k-fold or leave-one-out cross-validation, which prevents optimistic bias by separating model tuning from evaluation.
5. **External Validation.** Performance is estimated using hold-out or blinded validation sets.
6. **Refinement.** As new data become available or as additional biomodules mature, the model is revisited. Feature sets may be updated, architectures adjusted, and new validation runs performed. Predictors evolve with evidence, ensuring they remain both state-of-the-art and clinically reliable.

4.2 Technical implementation of biomarker products

When a predictor developed with krasID matures, it is packaged together with its dependencies into a phenomarker product, a ready-to-use, end-to-end software module that converts raw transcriptomic data into actionable predictions. Typically, biomarker products are delivered as a Python package with a command-line interface or as a Docker container for easy deployment.

A biomarker product typically includes the following components (*Figure 8*):

- **Input handling:** Accepts raw RNA-seq data or preprocessed expression matrices.
- **Preprocessing:** Performs normalization, batch-effect correction, and drift monitoring according to a proprietary harmonization system to ensure consistency across datasets.
- **Biomodule scoring:** Projects expression data into biologically interpretable biomodule scores using a subset of the Genialis Supermodel.
- **Predictor:** A trained ML model that generates categorical predictions (e.g., Responder/Non-Responder) or continuous predictions (e.g., time-on-treatment), probability scores, and optional covariate-adjusted outputs (such as dose consideration). Results can be provided in a standalone report or formatted for downstream analysis.



▲ FIGURE 8

Each krasID predictor can be bundled with all necessary dependencies into a standalone software package that accepts RNA-seq data as input and produces krasID scores with a single command.

Call to collaborate

If you are developing a KRAS inhibitor or a combination strategy, Genialis invites you to explore how krasID can accelerate your program.

The first step is simple: partner with us to train and test a predictor on your data. From there, we will help refine it into a clinical phenomarker that differentiates your compound and ensures approval of your drug.

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Genialis

Know your patient
Know your drug

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