Predictive and Generative AI to Guide the Clinical Development of HFB200301, a First-in-Class TNFR2 Agonist: Drug Intelligence Science (DIS®)

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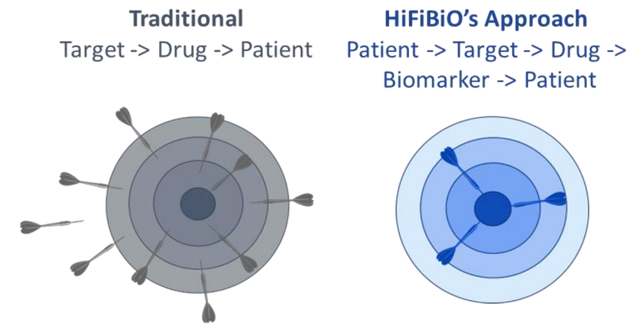
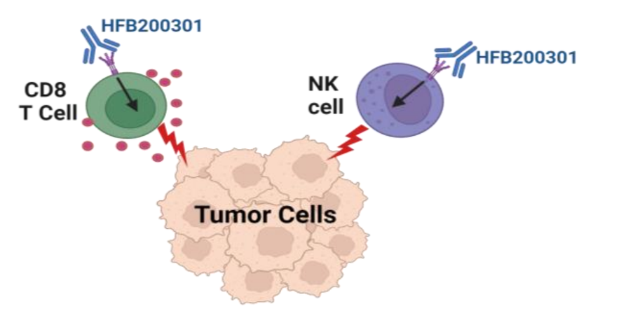
BACKGROUND

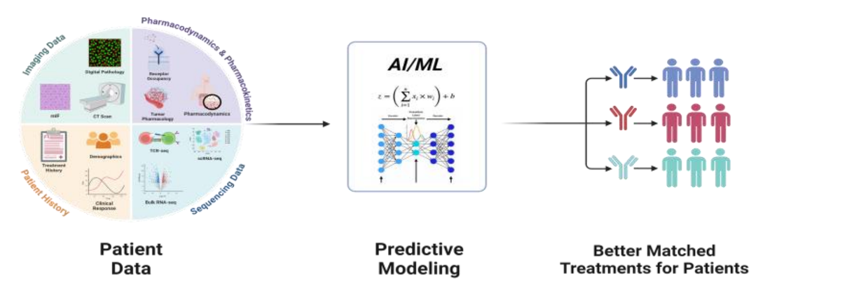
- HiFiBiO has developed an innovative approach to drug development through its Drug Intelligence Science (DIS®) platform, which leverages the power of multi-modal artificial intelligence (AI). By integrating both predictive and generative models, the DIS® platform is designed to:
  - Precisely match subjects with the most suitable therapies
  - Accelerate the drug development timeline
  - Enhance the probability of success in clinical trials



- The DIS® platform has been applied to select tumor types most likely to respond to HFB200301, a first-in-class agonistic monoclonal antibody targeting TNFR2. This is being evaluated in an ongoing Phase 1 clinical trial (NCT05238883).



First-in-class TNFR2 agonist HFB200301 MoA



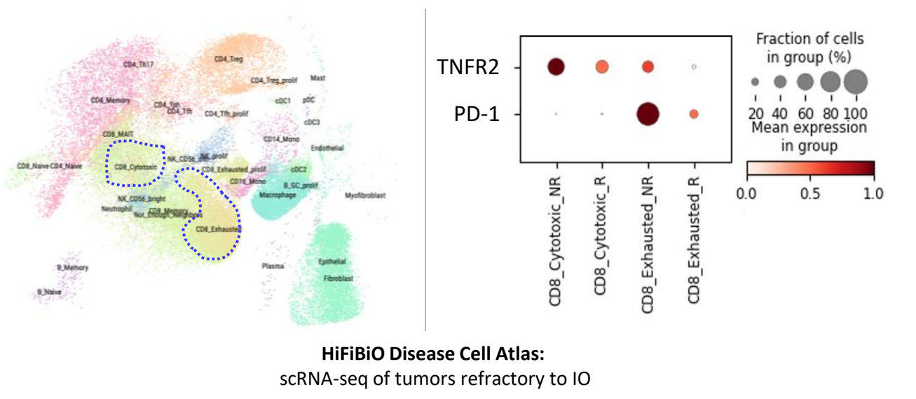
HiFiBiO's Drug Intelligence Science (DIS®) Platform to match the right subjects with the right treatments

- At the core of the DIS® platform is the **HiFiBiO AI Response Prediction (HARP)** tool, which integrates real-time biomarker and clinical data from the ongoing Phase 1 clinical trial. HARP aims to refine the predictive models and continuously optimize indication selection.

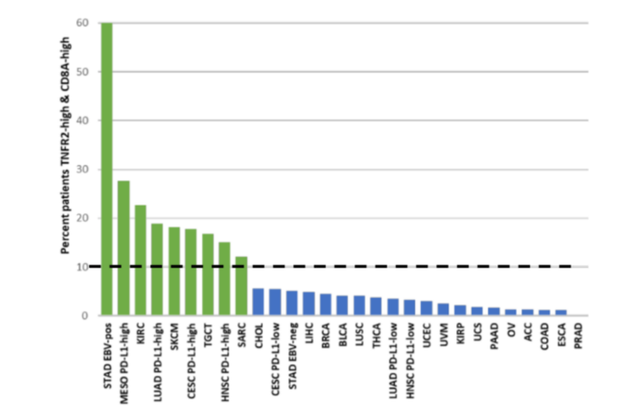
HFB200301 PHASE 1 TRIAL

DIS® Selected Tumor Types

- **SINGLE-CELL DATA** show TNFR2 highly expressed on CD8+ T cells lacking PD-1 in tumors resistant to anti-PD-(L)1.

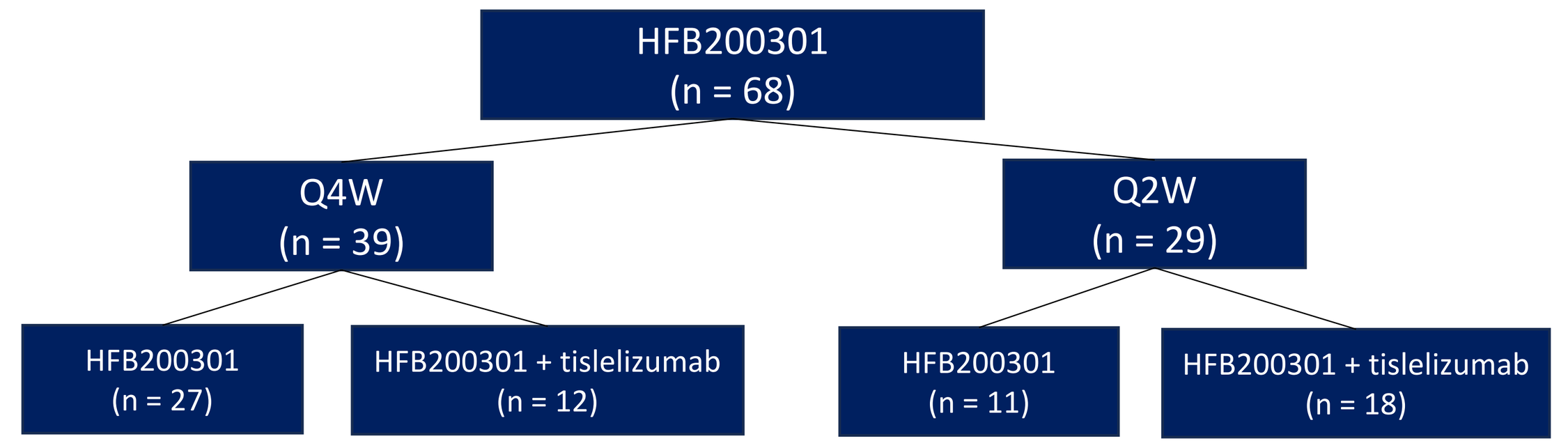


- Prior to Phase 1, DIS® identified nine tumor types using TNFR2 and CD8A expression in bulk RNA expression databases.



HFB200301 Trial Summary and Clinical Benefit Criteria

- **THE TRIAL** is evaluating HFB200301, a first-in-class TNFR2 agonist, in 68 subjects diagnosed with advanced, treatment-resistant tumors. The trial includes two dosing regimens: 39 subjects receive the treatment every four weeks (Q4W), while 29 subjects are treated every two weeks (Q2W).



- **CLINICAL BENEFIT UPON HFB200301 TREATMENT**, was defined as having either i) a cumulative decrease in target lesion size of 0% or more from baseline, or ii) being on HFB200301 treatment for > 6 months.

At the time of this report, 9 subjects met one or both of these criteria.

AI-READY CLINICAL DATABASE & HARP INTEGRATED MULTI-MODAL DATA FOR PREDICTIVE BIOMARKER DISCOVERY

HFB200301 Biomarker Data Sets

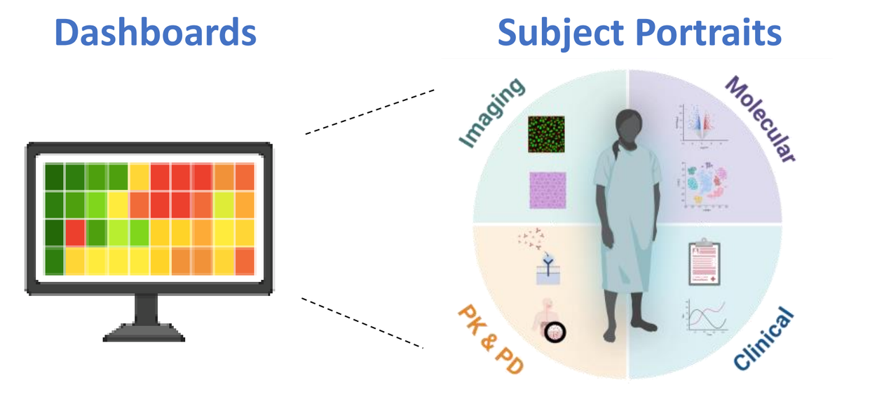
- **Subject Data Collection** is summarized in the table below.

Data Modality	Subject Sample Set	
	Clinical Benefit (N at Screening)	No Clinical Benefit (N at Screening)
Tumor bulk RNA-sequencing (bulk RNA-seq)	3	18
PBMCs single-cell RNA-sequencing (scRNA-seq)	3	17
Clinical assessments & pharmacodynamic markers	9	54

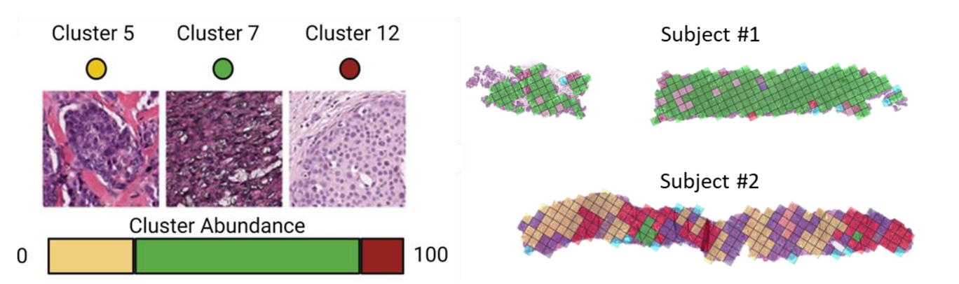
Note this table only includes subjects with known clinical benefit. The specific subjects may or may not overlap across biomarker modalities.
The HFB200301 trial is still ongoing and the response status for some subjects is unknown.

Dashboards for Real-Time Clinical Monitoring and Subject Portrait Generation

- **Real-time Dashboards** accelerate the transition from data collection to actionable insights, facilitating clinical monitoring and faster biomarker discovery.
- **Deep Learning** generates precise patient features from HiFiBiO dashboards for predictive AI models that enhance decision making for improved clinical success.
- **Subject Portraits** integrate multi-modal biomarker and clinical data to create comprehensive profiles.
- **Augmented Subject Portraits** can be generated by integrating data from public databases. To illustrate this, we developed a deep learning digital pathology model, trained with Hematoxylin and Eosin (H&E) whole-slide tumor images from relevant indications, and identified 16 distinct morphological clusters in the tumor biopsies from HFB200301 trial subjects.



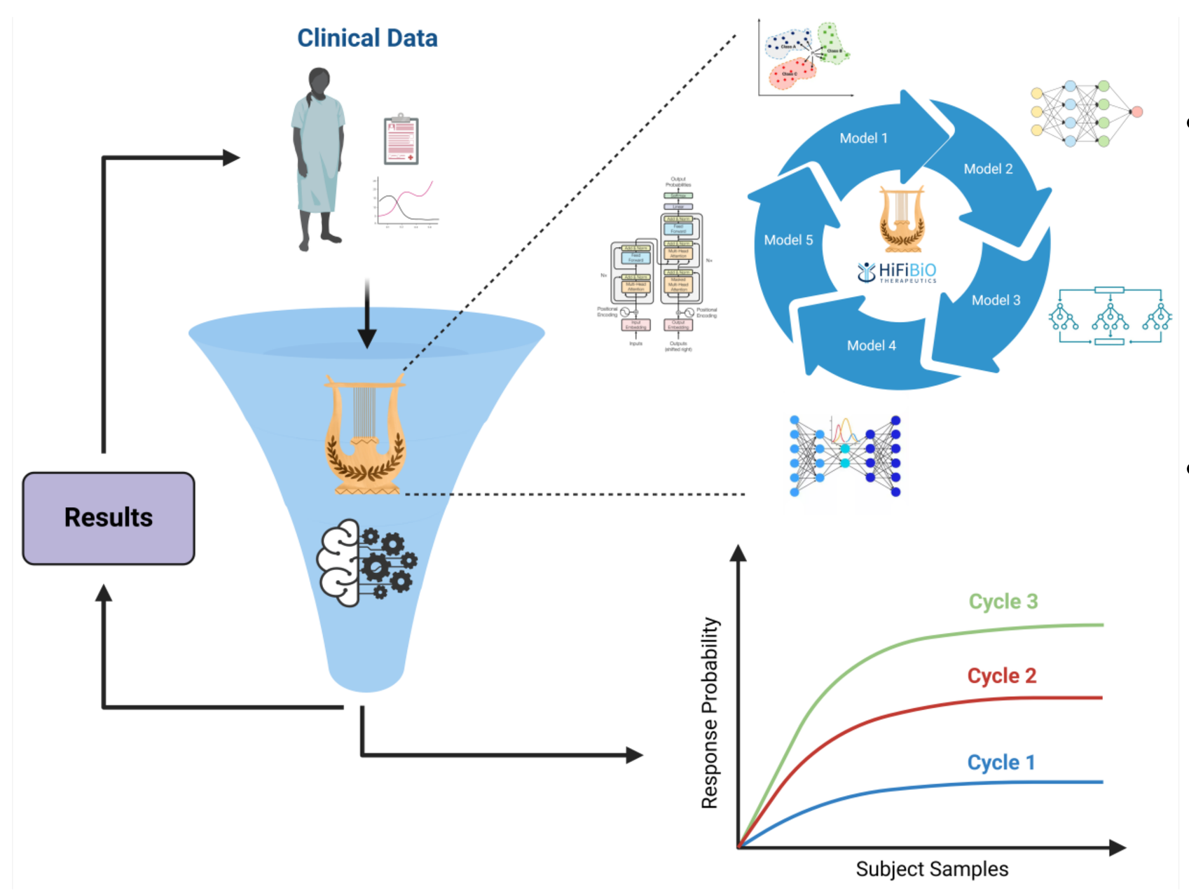
Morphology Cluster Assignment Using Digital Pathology



H&E tiles in the schematic are not representative of Clusters 5, 7, or 12. They are used only for illustrative purposes.
Distinct morphological clusters were discovered from public datasets (n = ~1k) using deep learning and subsequently identified and projected into HFB200301 samples.

Representative example images

HARP: HiFiBiO AI Response Prediction

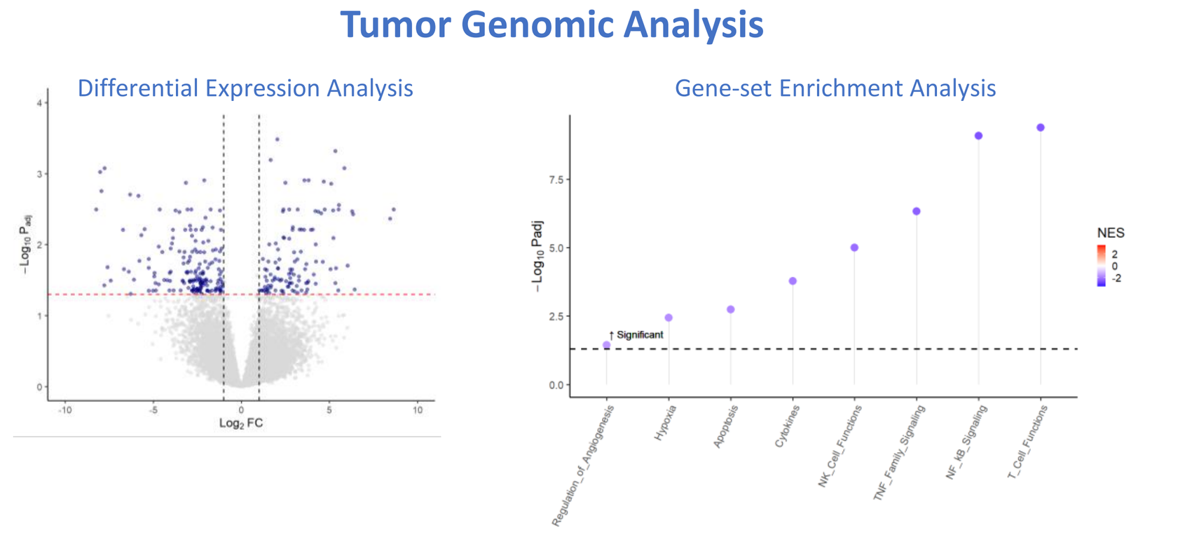


- **HARP** utilizes machine learning and AI to associate biomarkers with clinical benefit and refines patient selection.
- **Iterative Learning** improves HARP's predictions with real-time study results.

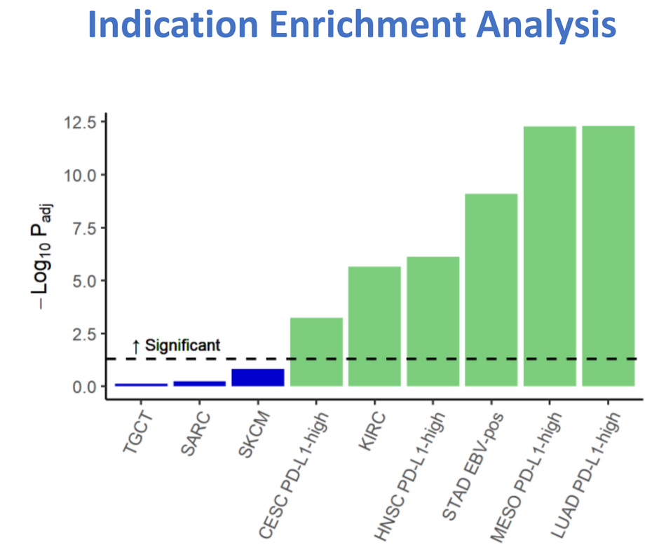
HFB200301 Tumor Signature Refines Indication Selection in Bulk RNA-seq Data

- **HARP IDENTIFIED A GENE SIGNATURE** in the baseline/screening biopsies of tumors from subjects benefiting from HFB200103 treatment (n=3) compared to tumors from subjects with no clinical benefit (n= 18). This signature was enriched for gene-sets related to NK and T cell functions, as well as TNF/NFkB signaling.
- **PROJECTION OF THE TUMOR SIGNATURE** into bulk RNA expression databases revealed enrichment of the HFB200301 Tumor Signature in six of the nine selected indications, which were prioritized for further investigation.

Tumor Genomic Analysis



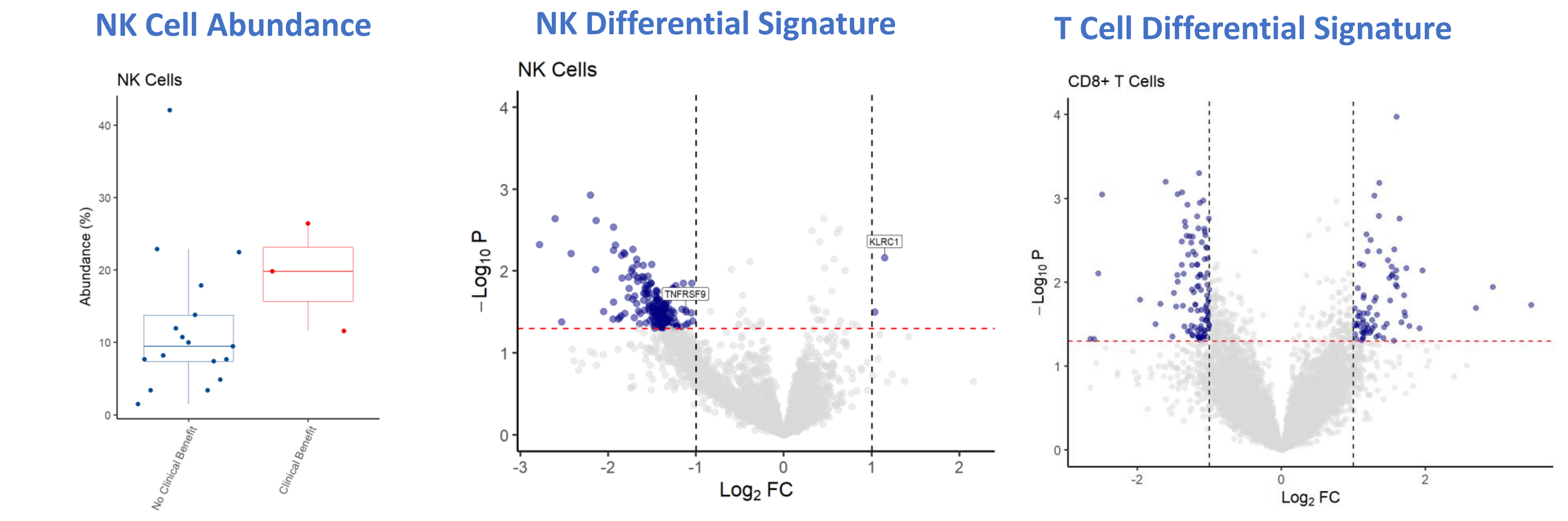
Indication Enrichment Analysis



bulkRNA-seq data processed and cleaned with proprietary pipeline. NES = normalized enrichment score.

Peripheral NK/T Cell Changes Linked to HFB200301 Clinical Benefit in scRNA-seq Data

- Peripheral single-cell RNA-seq data reveals differential abundance of NK cells and differential transcription in NK and CD8+ T cells in subjects receiving clinical benefit versus no clinical benefit at baseline. Subjects receiving clinical benefit tend to possess inactive NK cells, as indicated by KLRC1 and TNFRSF9 expression, and possess a unique CD8+ T cell signature, which may serve as accessible biomarkers.

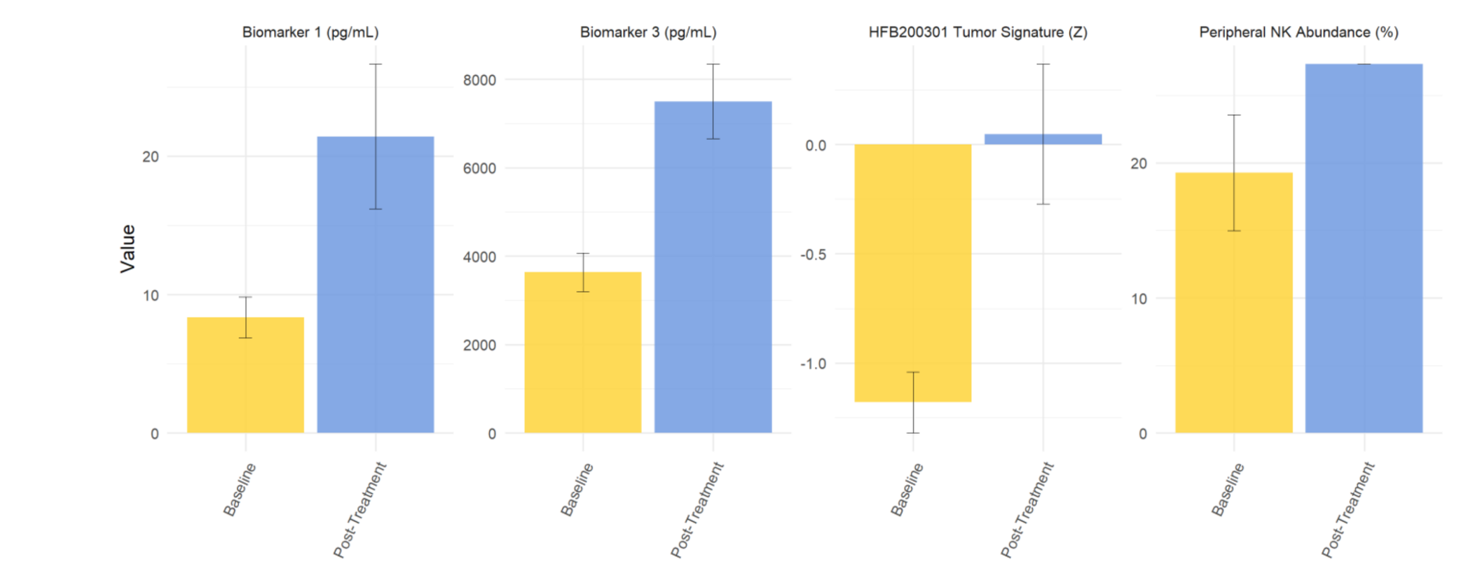


scRNA-seq data integrated with HiFiBiO generative AI; ~800k cells passing QC. Statistical methods employed Bayesian multi-level modeling.
Peripheral NK cell abundance was statistically different between subjects receiving clinical benefit vs no clinical benefit at baseline (p < .1).

HFB200301 Treatment Modulates Biomarkers in Tumor and Periphery

- **BIOMARKERS MODULATED BY HFB200301 TREATMENT** in longitudinal molecular and clinical data from both peripheral and tumor samples. These findings highlight that the clinical benefit markers are related to the mechanism of action for HFB200301.

HFB200301 Pharmacodynamic Effects in Subjects Receiving Clinical Benefit

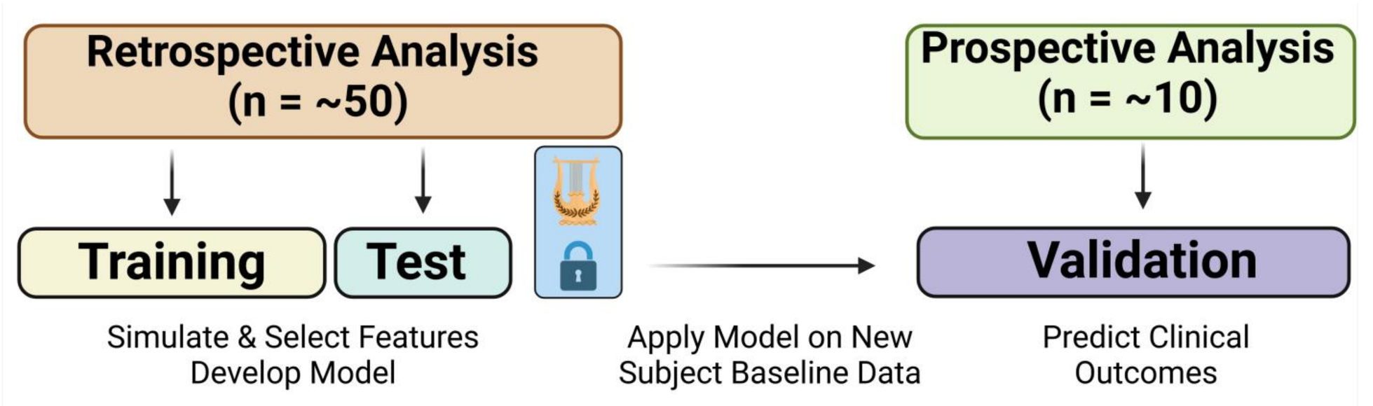


Barplots showing average +/se bars of baseline and post-treatment values of clinical benefit biomarkers in subjects receiving a clinical benefit to HFB200301.
Post-treatment timepoint is cycle 2 day 8 for HFB200301 Tumor Signature and peripheral NK abundance and is 24hrs after the first dose for Biomarker 1/3.Biomarker 2 had no longitudinal timepoint.
The # of post-treatment samples for subjects receiving clinical benefit are 7, 8, 4, and 1 for Biomarker 1, Biomarker 3, HFB200301 Tumor Signature, and peripheral NK abundance, respectively

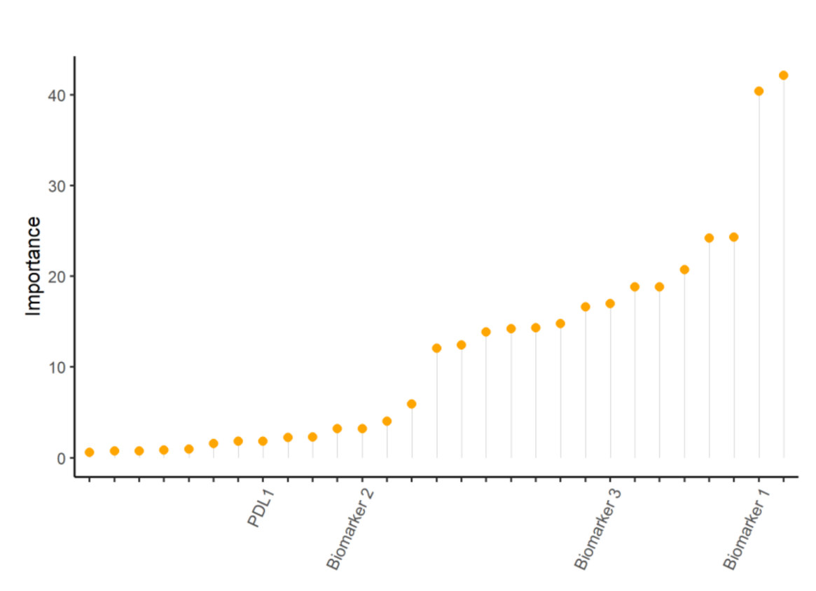
PREDICTING CLINICAL BENEFIT IN REAL-TIME WITH HARP

HARP Identifies Features Potentially Predictive of Clinical Benefit

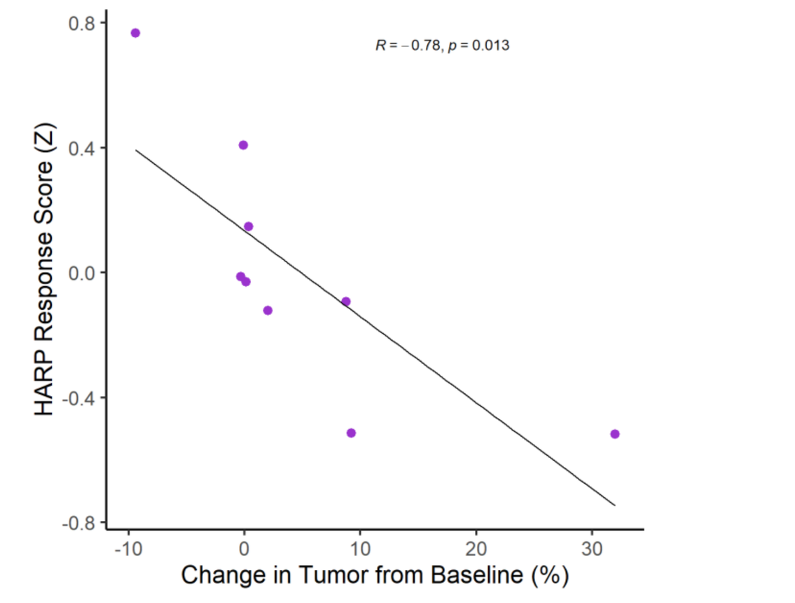
- Utilizing iterative simulations on subjects' clinical assessments, our proprietary machine learning model, HARP, identified biomarkers associated with clinical benefit to HFB200301 treatment.
- The HARP model, trained on retrospective clinical data, was applied to baseline data from subjects without prior tumor response information. Each patient was assigned a HARP response score, which was later correlated with tumor reduction following HFB200301 treatment.



Feature Selection



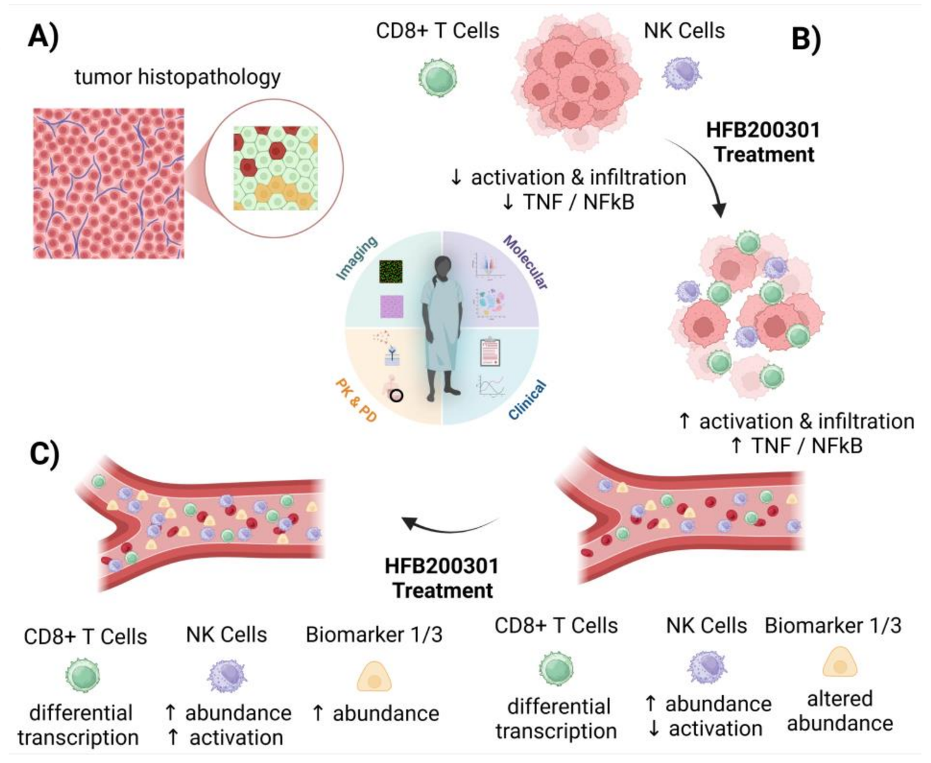
HARP Score Correlates with Tumor Reduction



Multi-modal subject data from HiFiBiO dashboards were aggregated, normalized, and pruned to remove redundant features.
Retrospective data were split into training and test sets, and leave-one-out cross-validation was performed.
The model was applied prospectively to subjects without prior tumor response data, and the resulting HARP scores were later correlated with tumor response.

SUMMARY & FUTURE DIRECTIONS

- By utilizing a suite of advanced machine learning models, we generated features associated with clinical benefit to HFB200301 treatment. This demonstrates the potential of leveraging generative and predictive artificial intelligence on multi-modal subject datasets to guide drug development.
- Our research confirms the mechanistic roles of various immune cells in mediating responses to HFB200301 and identifies novel biomarkers that correlate with clinical benefit (see schematic summary).
- Through comprehensive analyses of multi-modal subject data from both tumor and peripheral samples, we refined our indication selection to better match subjects with treatments and identified a variety of biomarkers that associated with clinical benefit to HFB200301 treatment.
- Building on these findings, we plan to validate these biomarkers in larger cohorts and refine our AI models to enhance predictive accuracy. Integrating these insights will inform the design of subsequent clinical trials, ultimately aiming to improve therapeutic outcomes for subjects treated with HFB200301.



A) Deep learning approaches generated histopathological features in tumor imaging data from HFB200301 subjects.

B) Bulk RNA-seq tumor data suggested decreased TNF/NFkB signaling and decreased activation of immune cell types (NK / T cells), which were increased treatment.

C) Multi-modal peripheral subject data identified altered abundance of NK cells, differential transcription of CD8+ T cells and clinical biomarkers levels, which correlated with clinical benefit to HFB200301 treatment at baseline and were modified by treatment.

Acknowledgments

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For additional information, please email contact@hifibio.com or visit HiFiBiO.com