



Background

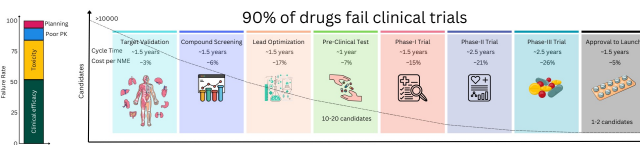


Fig. 1: Overview of clinical trials showing success rates and a strong decline in therapeutic candidates in each phase.

A PD-1/L1 mAb Clinical Trial Landscape

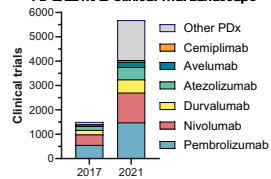
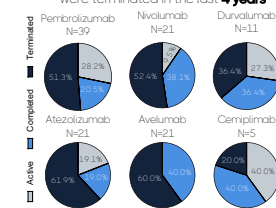


Fig. 2: (A) PD-1/PD-L1 immunotherapies have made significant progress in cancer treatment; however, (B) often face challenges in clinical trials.

B Approximately 50% of the clinical trials were terminated in the last 4 years



How Nilogen's 3D platforms lower risk of failure

	FOX Model	Organoid	Spheroid	Tumoroid
Physiological Relevance	LOW	NO/LOW	NO	HIGH
Translatability	LOW/MEDIUM	NO/LOW	NO	HIGH
Mutation Eff.	HIGH	HIGH	HIGH	LOW
ECM	NO	NO	NO	INTACT
Immune Microenvironment	NO	NO	NO	YES
Time	LONG	SHORT	SHORT	SHORT
Cost	> \$500,000	> \$200,000	> \$200,000	\$10,000

Fig. 3: Comparison of Nilogen's 3D-EX vivo Tumoroid Platform and Other Available Models for Testing Therapeutics. A comparative overview of FOX (Patient-Derived Xenograft) models and various ex vivo models (Organoids, Spheroids, Tumoroids) in terms of physiological relevance, translatability, mutation drift, extracellular matrix (ECM) presence, immune microenvironment, time, and cost. Nilogen's tumoroid platform exhibits the highest physiological relevance and translatability, with an intact ECM and the presence of an immune microenvironment, making it a valuable model for cancer research. In contrast, FOX models are costly, time-intensive, and have higher mutation drift, while spheroids and organoids lack the same level of complexity and relevance seen in tumoroids.

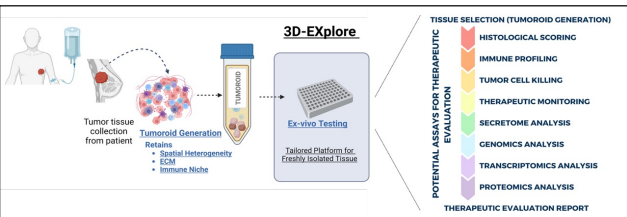


Fig. 4: Comprehensive Therapeutic Evaluation Framework. Workflow for evaluating therapeutics using the 3D-EXplore/Express platforms. Tumor tissue collected from patients is minimally processed to generate tumoroids, retaining spatial heterogeneity, ECM, and the immune niche. Ex-vivo testing is performed using the 3D-EXplore/Express platform, enabling various assays for therapeutic evaluation, including histological scoring, immune profiling, tumor cell killing, therapeutic monitoring, secretome, genomics, transcriptomics, and proteomics analyses. The results are compiled into a therapeutic evaluation report to guide clinical decisions.

Results

Meta-Analysis of Clinical Trials Evaluating Various Immunotherapies in NSCLC

Study	Year	Sample Size (n)	Treatment Regimen	PD-L1 Expression Criteria	Objective Response Rate (ORR)	Progression-Free Survival (PFS)	Overall Survival (OS)	Key Findings
KEYNOTE-024	2016	305	Pembrolizumab vs. Chemotherapy	PD-L1 ≥50%	41.8% (Pembro) vs. 27.8% (Chemo)	10.3 months (Pembro) vs. 6.0 months	9.0 months (Pembro) vs. 14.2 months	Pembrolizumab significantly improved OS and PFS compared to chemotherapy in PD-L1 high NSCLC.
KEYNOTE-049	2018	616	Pembrolizumab + Chemotherapy vs. Chemotherapy	PD-L1 ≥1% in All-Cohorts	47.6% (Pembro Combo) vs. 18.9% (Chemo)	8.8 months (Pembro Combo) vs. 4.9 months	NR (Not Reached) vs. 11.3 months	Combination therapy with pembrolizumab significantly improved outcomes in all PD-L1 groups.
KEYNOTE-042	2019	1274	Pembrolizumab vs. Chemotherapy	PD-L1 ≥1%	27% (Pembro) vs. 16% (Chemo)	7.7 months (Pembro) vs. 5.5 months	20 months (Pembro) vs. 12.2 months	Pembrolizumab monotherapy showed OS benefit in patients with PD-L1 ≥1%.
KEYNOTE-058	2021	568	Pembrolizumab + Ipilimumab vs. Pembrolizumab	PD-L1 ≥50%	48% (Combo) vs. 43% (Pembro)	9.7 months (Combo) vs. 9.8 months	NR (Combo) vs. NR (Pembro)	Combination did not significantly improve outcomes compared to pembrolizumab alone.
KEYNOTE-407	2018	559	Pembrolizumab + Chemotherapy vs. Chemotherapy	PD-L1 ≥1% in All-Cohorts	57.3% (Pembro Combo) vs. 38.4% (Chemo)	6.4 months (Pembro Combo) vs. 4.8 months	15.9 months (Pembro Combo) vs. 11.1 months	Significant improvement in OS and PFS with pembrolizumab combination therapy.
KEYNOTE-010	2015	1034	Pembrolizumab vs. Docetaxel	PD-L1 ≥1%	21% (Pembro) vs. 9% (Docetaxel)	3.9 months (Pembro) vs. 3.0 months	16.4 months (Pembro) vs. 8.5 months	Pembrolizumab was superior to docetaxel in OS and ORR, especially in PD-L1 ≥50%.
CheckMate 017	2015	272	Nivolumab vs. Docetaxel	No PD-L1 Criteria	20% (Nivo) vs. 9% (Docetaxel)	3.5 months (Nivo) vs. 2.8 months	12.2 months (Nivo) vs. 9.4 months	Nivolumab improved OS compared to docetaxel in previously treated NSCLC patients.
CheckMate 057	2017	492	Nivolumab vs. Docetaxel	No PD-L1 Criteria	16.6% (Nivo) vs. 12.6% (Docetaxel)	5.1 months (Nivo) vs. 3.0 months	12.6 months (Nivo) vs. 9.7 months	Nivolumab demonstrated improved overall outcomes compared to docetaxel.
CheckMate 227	2019	1,460	Nivolumab + Ipilimumab vs. Chemotherapy	PD-L1 ≥1% in All-Cohorts	48% (Combo) vs. 27% (Nivo)	7.2 months (Combo) vs. 5.8 months	NR (Combo) vs. 10.7 months	Combination therapy significantly improved PFS and OS compared to chemotherapy.
Ipilimumab 050	2019	1,202	Atezolizumab + Chemo vs. Atezolizumab + Chemo	PD-L1 ≥1%	56% (Atezolizumab Combo) vs. 47% (Ipilimumab Combo)	8.3 months (Atezolizumab Combo) vs. 6.8 months	19.2 months (Atezolizumab Combo) vs. 14.7 months	Atezolizumab combination therapy provided improved survival outcomes in NSCLC patients.
CheckMate 34L	2020	713	Nivolumab + Ipilimumab vs. Chemo vs. Chemo	No PD-L1 Criteria	51% (Combo) vs. 38% (Chemo)	NR (Combo) vs. 6.1 months	NR (Combo) vs. 10.1 months	Nivolumab + Ipilimumab + chemotherapy improved PFS and OS in NSCLC.

Table 1: Overview of significant clinical trials assessing different immunotherapeutic agents in non-small cell lung cancer (NSCLC). Each entry includes the study name, publication year, sample size, treatment regimens, PD-L1 expression criteria, objective response rates (ORR), progression-free survival (PFS), and overall survival (OS). The results highlight the efficacy of various immunotherapies, including pembrolizumab, nivolumab, atezolizumab, and combination therapies, in improving patient outcomes.

NSCLC Responder Analysis

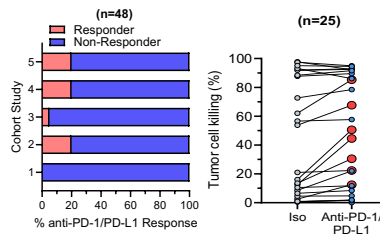


Fig. 7: (A) Proportions of NSCLC responders (red) and non-responders (blue) to anti-PD-1/PD-L1 therapies across five cohort studies. (B) Tumor cell killing in NSCLC samples (N=25) with isotype control (Iso) and anti-PD-1 treatment. Lines connect paired samples, with red circles for responders and blue for non-responders. Circle size reflects the magnitude of cell killing.

Gene Signature Analysis

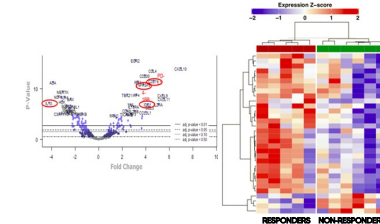


Fig. 8: Gene expression profiling analysis using Nanostring IO360. (A) NSCLCs tumors treated with Pembrolizumab displaying increased expression of lymphoid and myeloid expression marker. (B) Gene expression profile between "Responders" and "Non-Responders" displayed robust differences.

Basket Responder Analysis

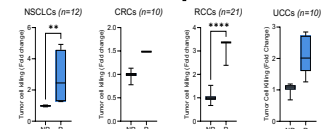


Fig. 5: The fold change in tumor cell killing for non-responders "NR" vs responders "R" after treating tumoroids with Pembrolizumab for 48-72hrs. Data derived from 12 NSCLC, 10 CRC, 21 RCC, and 10 UCC tumoroid tissues. The data was normalized to isotype controls for both the groups and presented as box and whisker plot with box representing Q2 and Q3 with median value dissecting both quartiles. The whiskers ranges as 5-95 percentile of data. A two-tailed student t-test (non-parametric) was performed for comparison between the two groups. **p < 0.01, ***p < 0.001.

Basket Secretome Analysis

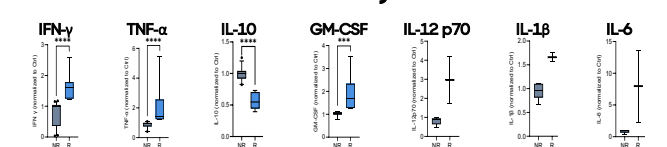


Fig. 6: The cytokine profiles of non-responders "NR" vs responders "R" after treating tumoroids with Pembrolizumab or isotype control for 48-72hrs. Data derived from 20 NSCLCs, 5 PDACs, 11 UCCs, 8 CRCs, and 12 RCCs tumoroid tissues. The data was normalized to isotype controls for both the groups and presented as box and whisker plot with box representing Q2 and Q3 with median value dissecting both quartiles. The whiskers ranges as 5-95 percentile of data. A two-tailed student t-test (non-parametric) is performed for comparison between the two groups. ***p < 0.001, ****p < 0.0001.

Nilogen 3D Platform Application in Clinical Trial Risk Assessment

- Predictive Modeling**
 - Offers better predictive outcomes for drug responses compared to traditional 2D cultures, leading to more reliable preclinical data.
 - Provides insights into tumor heterogeneity and microenvironmental influences on treatment responses.
- Preclinical Risk Evaluation**
 - Assists in the identification of safety concerns and potential side effects through rigorous testing in 3D models, reducing the likelihood of unexpected adverse events in clinical trials.
 - Provides a platform for biomarker discovery, allowing for the stratification of patients based on predicted responses to therapy.
- Patient-Centric Models**
 - Enables the risk assessment by simulating individual patient responses to treatments, thus guiding personalized therapy choices.
 - Supports adaptive trial designs by allowing for ongoing risk assessment throughout the trial based on real-time data.