



The menace of Cancer Metastasis:

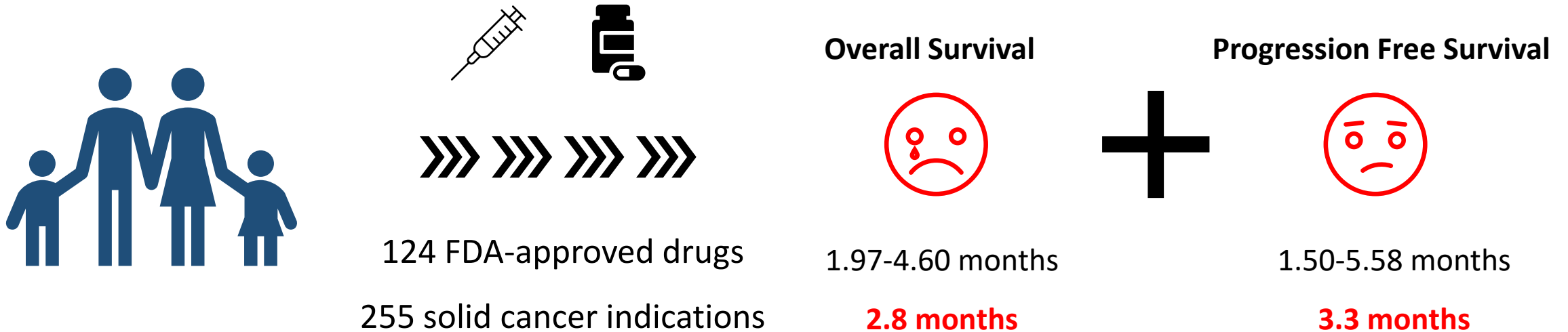
How do we fight it?

**Arnab Roy Chowdhury, PhD,
Founder / Director**

December, 2022

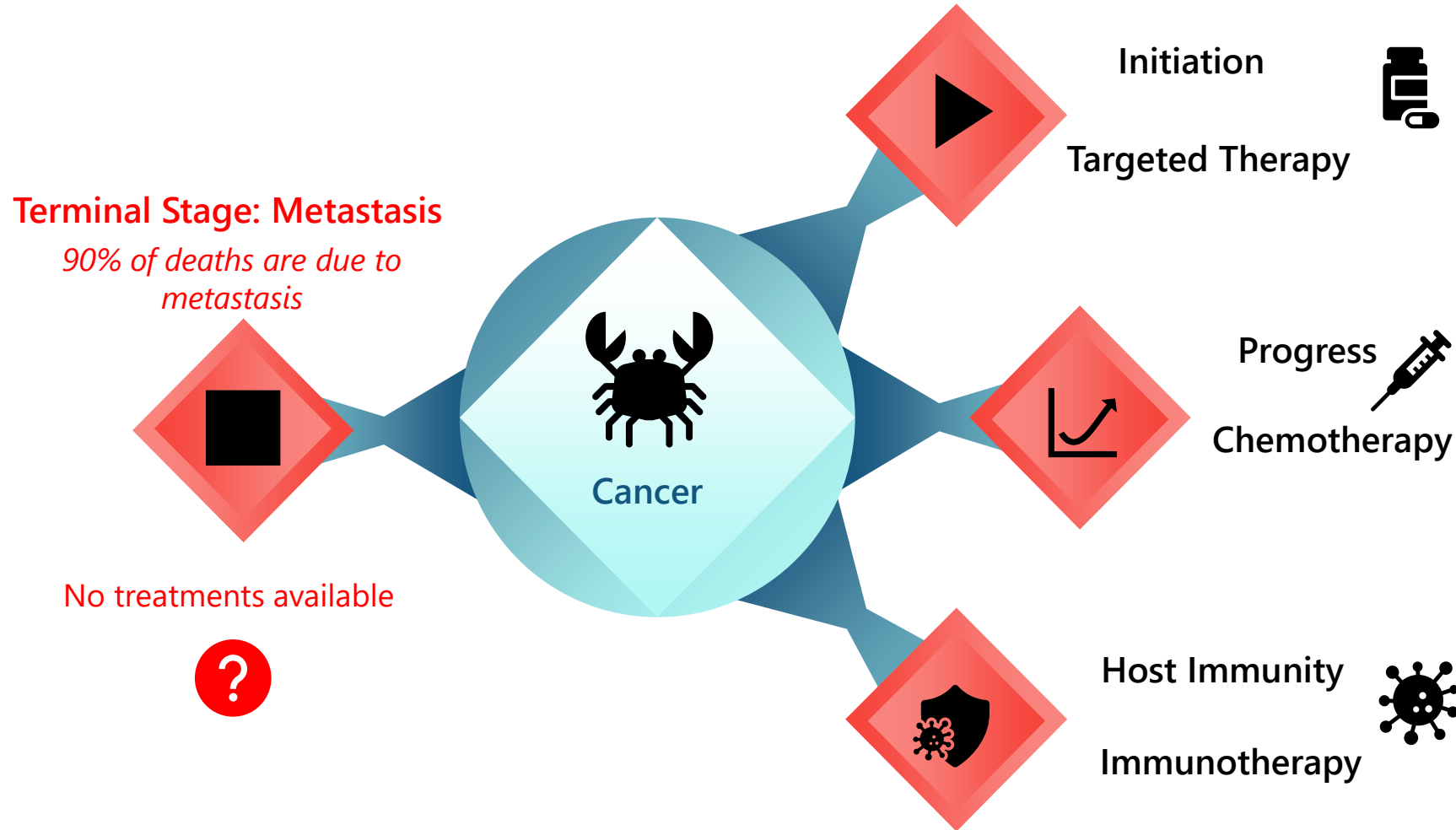
Bengaluru, India / Marlton, USA

Effect of new cancer drugs 2003-2021



J Clin Oncol. 2022 Dec 10; 40(35):4095-4106.

Numbers do not lie – cancer treatment is skewed



Approved drugs for
metastatic conditions
treat the secondary
tumour – **NOT** the
process of
metastasis

94

5-yr. survival of localized cancer
(breast-colon-prostate-melanoma)

21

5-yr. survival of metastatic cancer
(breast-colon-prostate-melanoma)

46

5-yr. survival of localized cancer
(pancreas-lung-liver-esophageal)

4.5

5-yr. survival of metastatic cancer
(pancreas-lung-liver-esophageal)

1

Approved drug for metastasis in last 10
years (Denosumab)

0

Blockbuster cancer drugs treat
metastasis

3-key challenges Impeding drug discovery

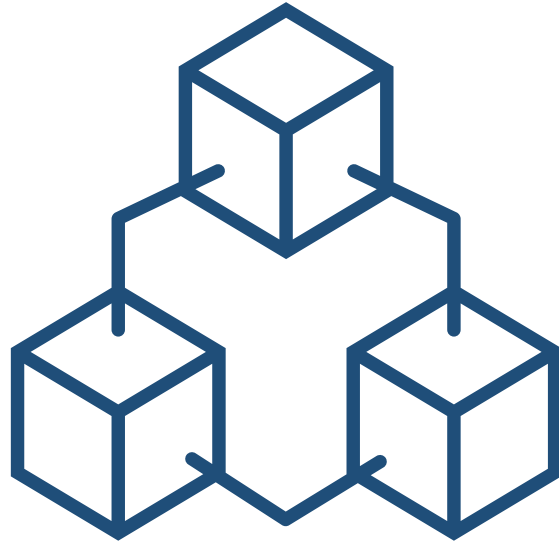
- 1 What is the **IDEAL** target that would translate into patients?
- 2 How to **TRIAGE** from 1000 to 1 molecule?
- 3 How to **SELECT** patients for clinical trials?

3 platforms to solve the puzzle

METAssay™



- 30 cellular assays dissecting metastasis
- How moving cells are different from growing cells



METSCAN™

METVivo™

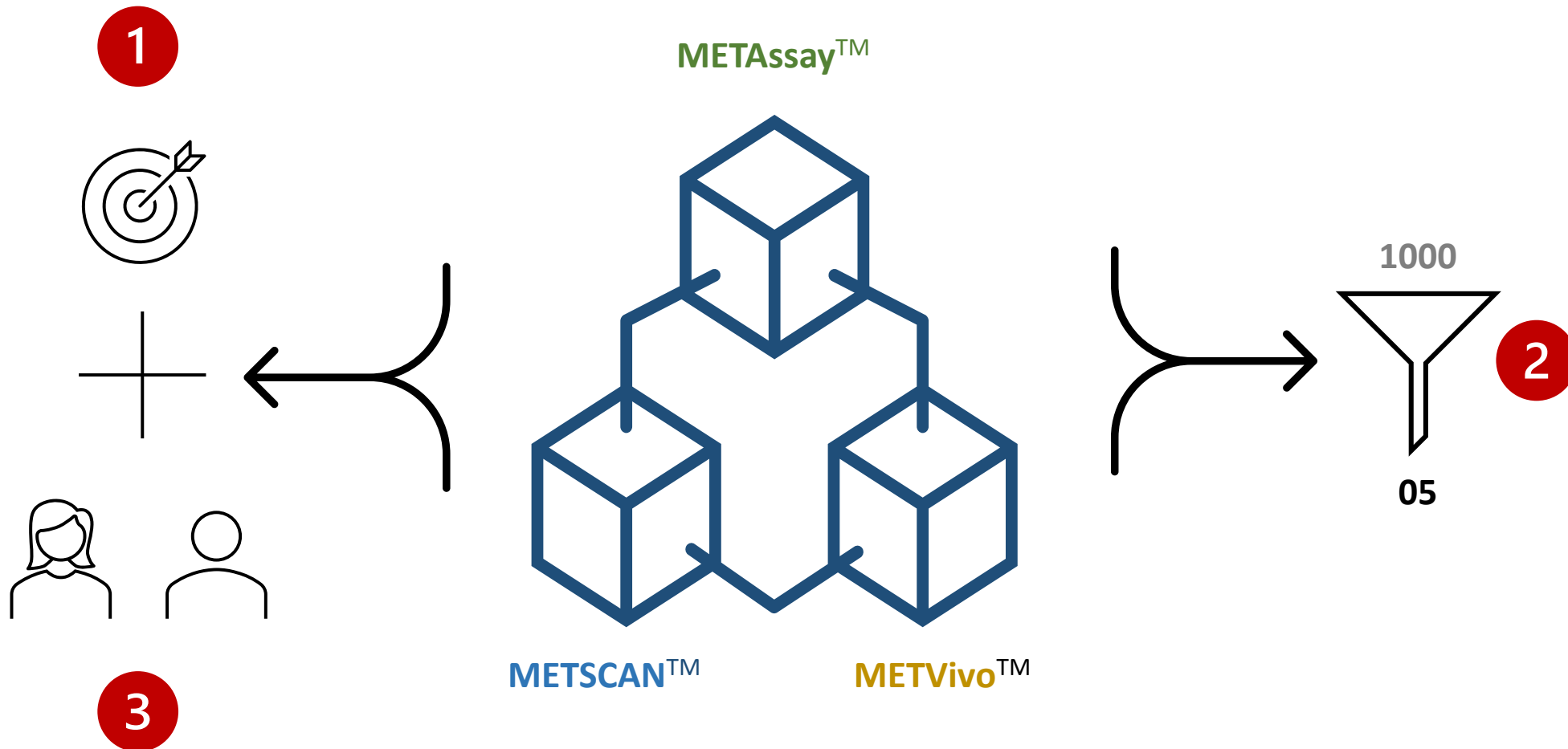


- Patient sample translation
- Which steps are most critical
- Which patients are at the highest risk



- Time-sensitive animal model
- Faster turnaround
- Robust, 100% met
- Kinetic and end-point

Our Solution



Our Capabilities

- Give us an **anti-cancer lead/candidate** – we will help position for **metastasis**
- Give us a **library of compounds**, and we will pick the best 5 with anti-metastasis activity
- Give us **approved drugs**, and we will repurpose the best compound for metastasis AND/OR profile them for **safety**
- Interested in any **tissue-specific** cancer or metastasis? We can help understand biology by **customizing** our platform.

Publications, Patents & Awards

8 international
posters to date



A cell-based phenotypic assay platform for cancer metastasis drug discovery and diagnostics

Dissecting tumorigenesis and metastatic properties of cell lines by phenotypic functional assays and plasticity ratio (PR)



The role of plasticity ratio (PR) in differentiating between metastatic and tumorigenic properties in cell lines and patient tumor samples



Tumor cell induced platelet aggregation is independent of tumor invasiveness as observed from both cell lines and primary tumors - 2021

METSCAN, a novel and proprietary algorithm to predict the metastatic potential of primary tumour patients - Nov 2022

COLLABORATORS



AWARDS

- Top 20 Biotech – India, 2021
- Top 10 R&D Startup – India, 2021
- Startup of the year in Biotech – India, 2021
- Top 20 Leading Healthcare Service Providers, India, 2022
- Winner - NITB Startup Expo, 2022
- Best Metastasis R&D specialists - GHP Global, 2022
- 3rd - IIT BHU Shark Tank, 2022
- Top 10 Healthcare, India, 2022
- Top 10 Biotech, India, 2022
- Next Generation presentation, Bio Eur 2022

PATENTS

PCT application published "Systems and methods for predicting cancer metastasis and screening of drugs"
#WO 2022/059026 A1

PCT filed "Methods for producing a spontaneous metastasis model"
#PCT/IN2022/050928



A novel, orthotopic spontaneous metastasis animal model for drug discovery that works in only six weeks



Survival and colonisation axis of metastasised cells in the secondary tissue: to target or not to target?

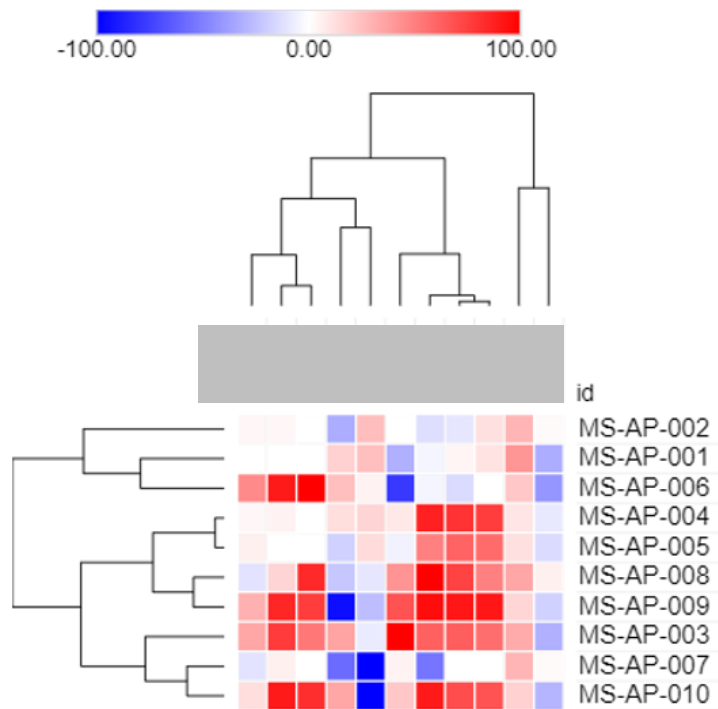


Exploiting the differential cellular biomechanics between metastatic and non-metastatic cells as a tool for predictive diagnostics

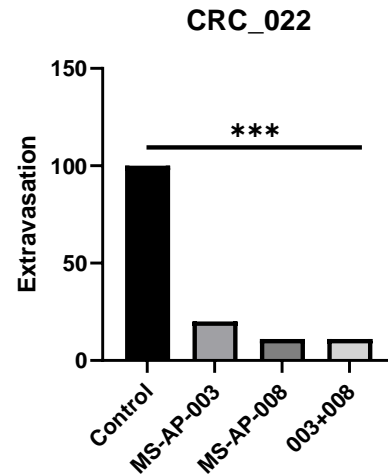
2 PCT filed

Case Study I: Triaging and Repurposing

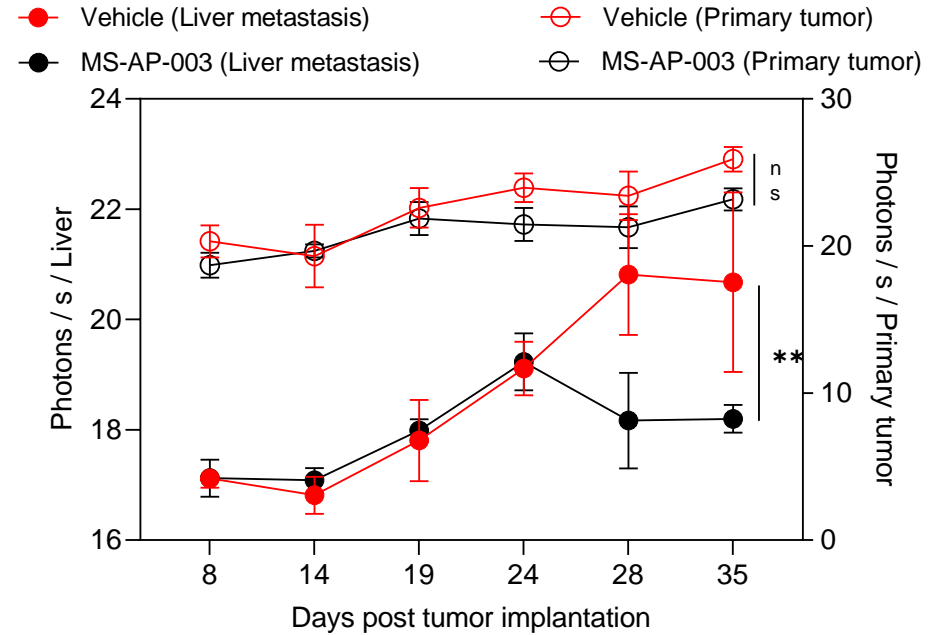
Case Study 1: Does the platforms work?



10 approved drugs with no reported anti-metastasis effect screened across the weighted platform

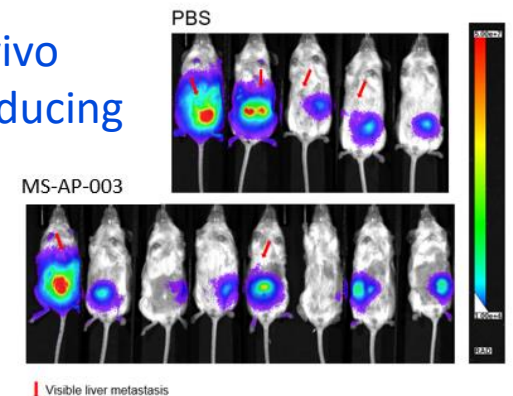


2 hits were progressed for multiple patient sample translational analysis



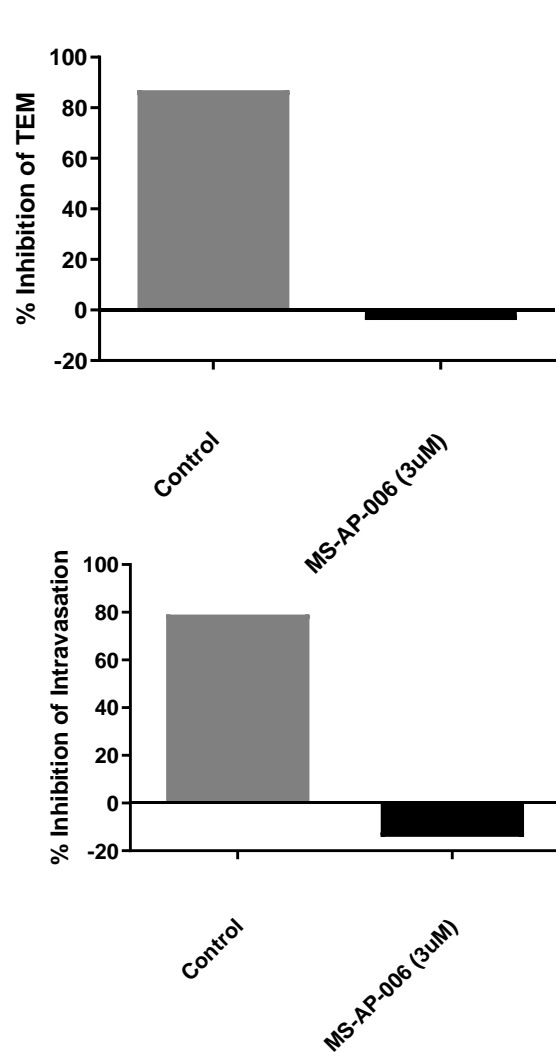
Excellent in vivo efficacy in reducing metastasis

No effect on tumour size

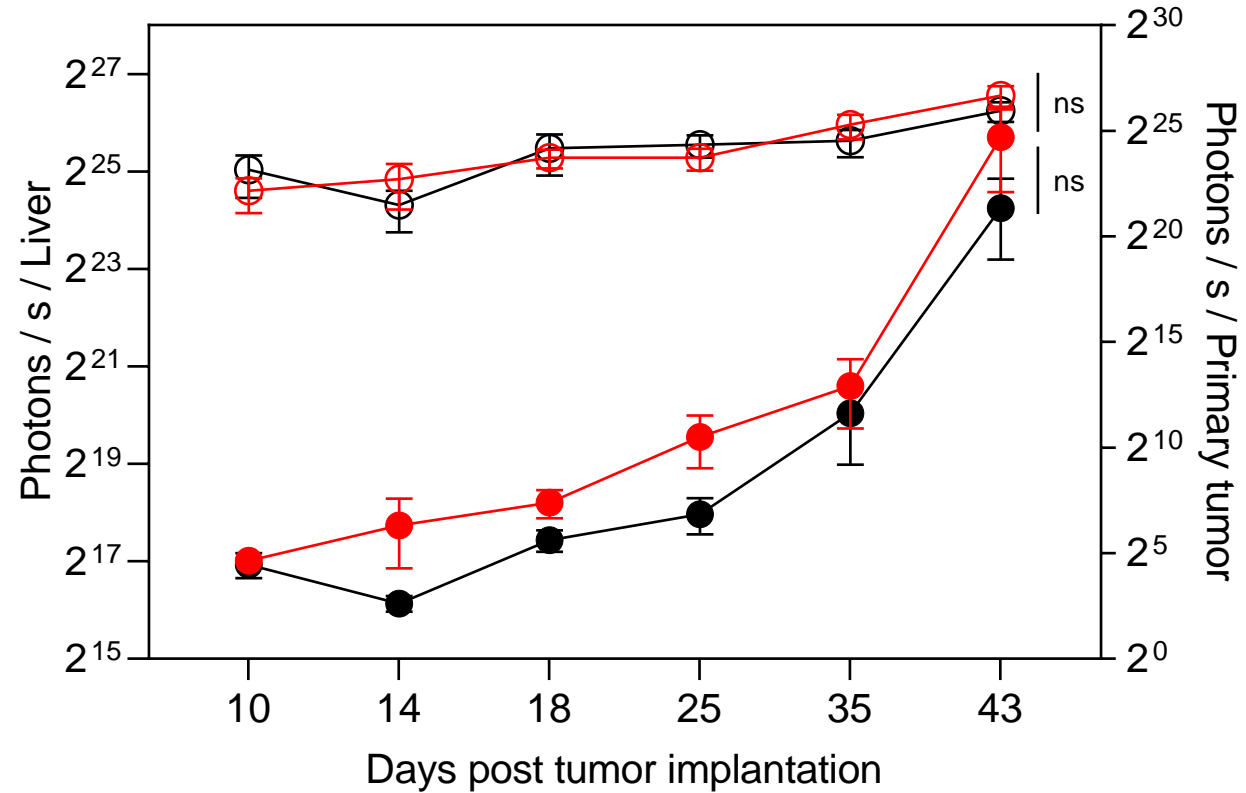


Visible liver metastasis

Negative Control establishes that platform is translational



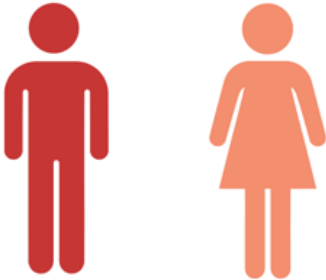
- Vehicle (Liver metastasis)
- AP-006 (Liver metastasis)
- Vehicle (Primary tumor)
- AP-006 (Primary tumor)



Clinical relevance of repurposing - Retrospective Study PoC

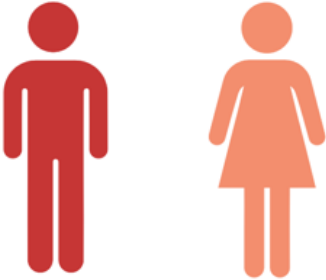


Colorectal tumors isolated from treatment naive primary tumor patients with no clinical metastasis*



Sep 2011 - Sep 2016

Follow - up on patients*

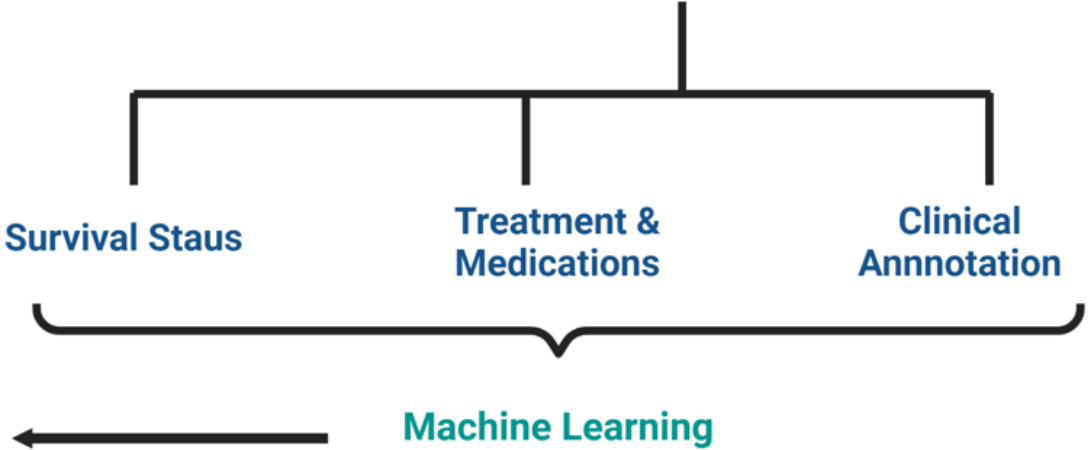


Sep 2022

Identify most **weighted factors** contributing towards **survival** and **death**



Effect of **medications**, treating **chronic diseases**, on **survival**

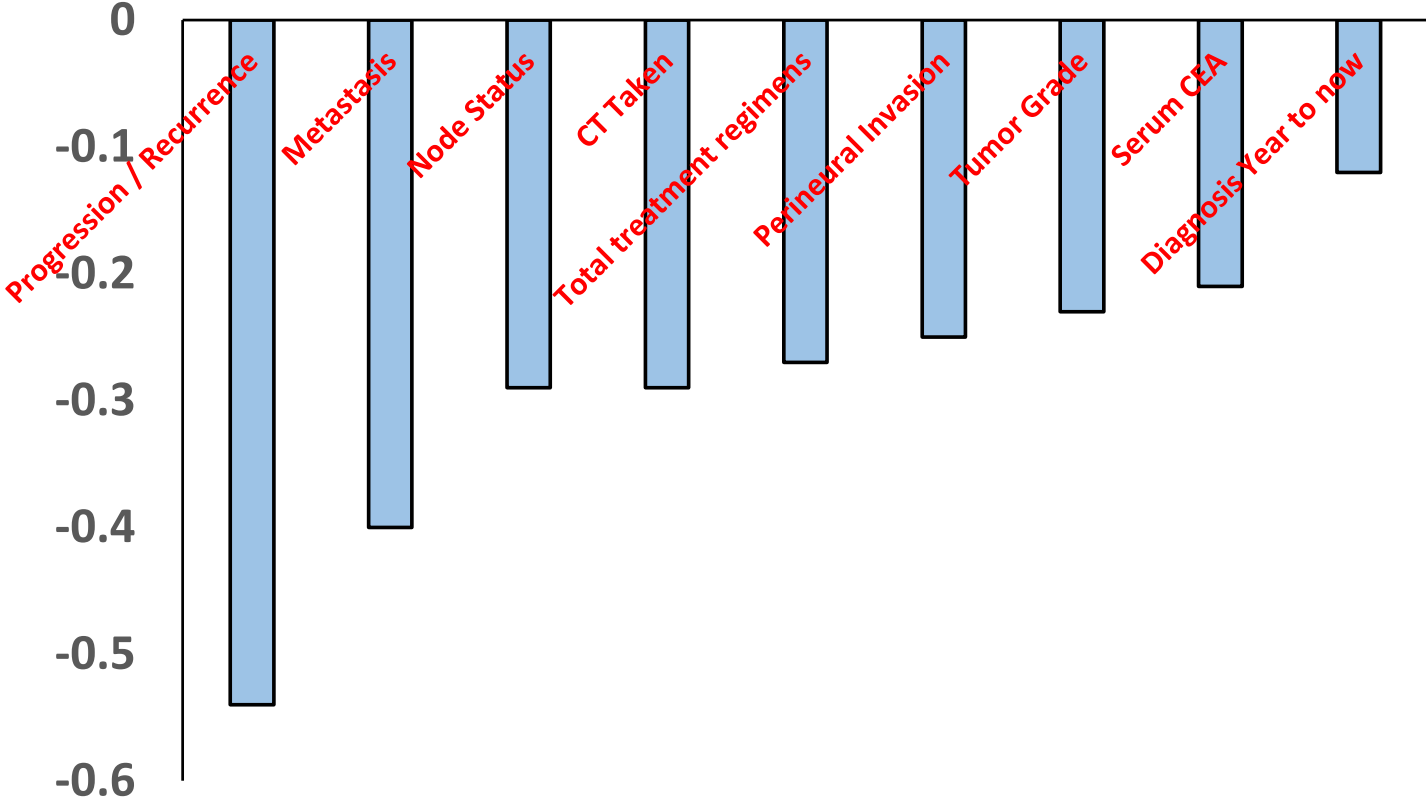


*In collaboration with Rajiv Gandhi Cancer Institute, Delhi

Most weighted steps, inversely affecting survival



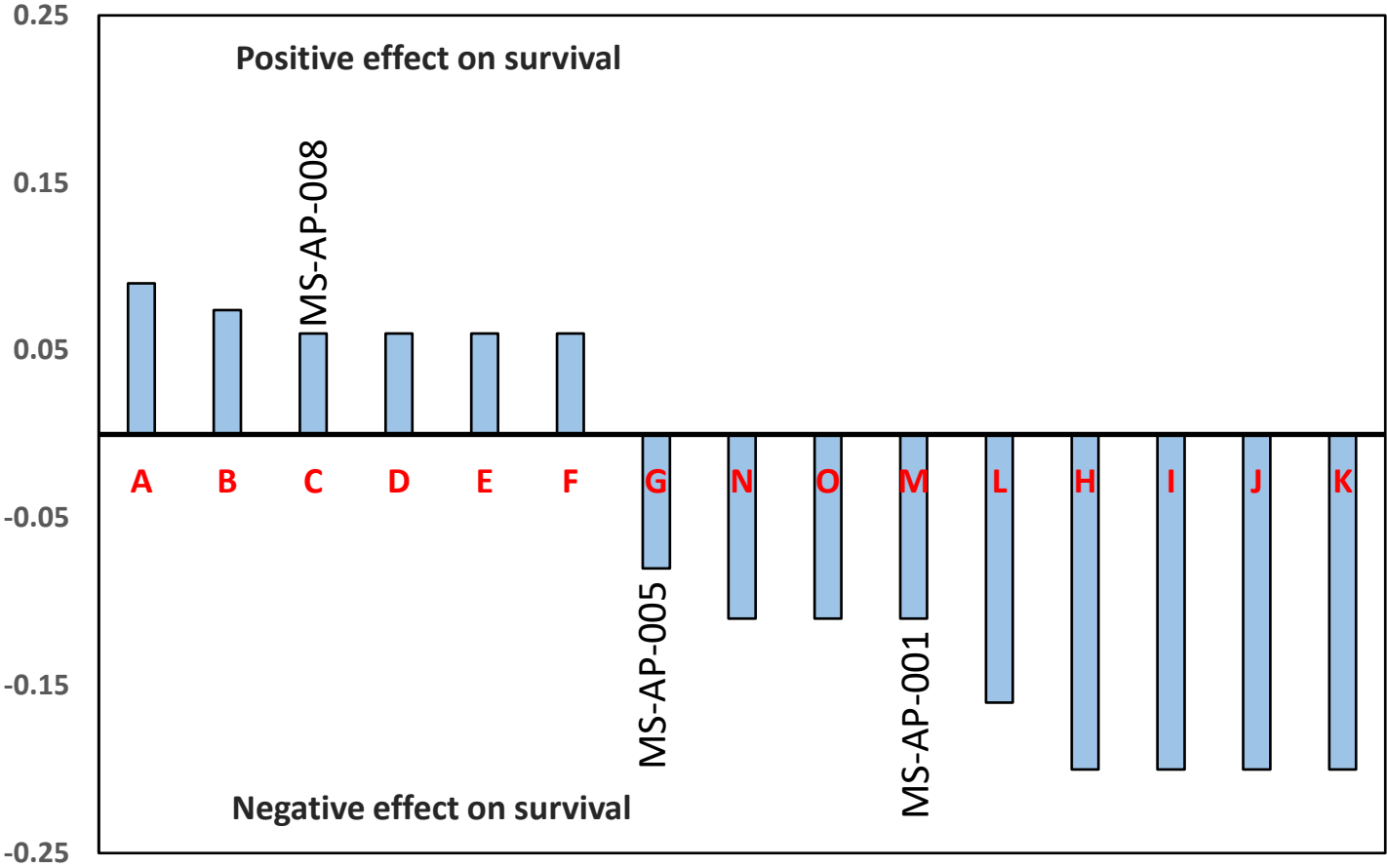
Negative correlation with survival



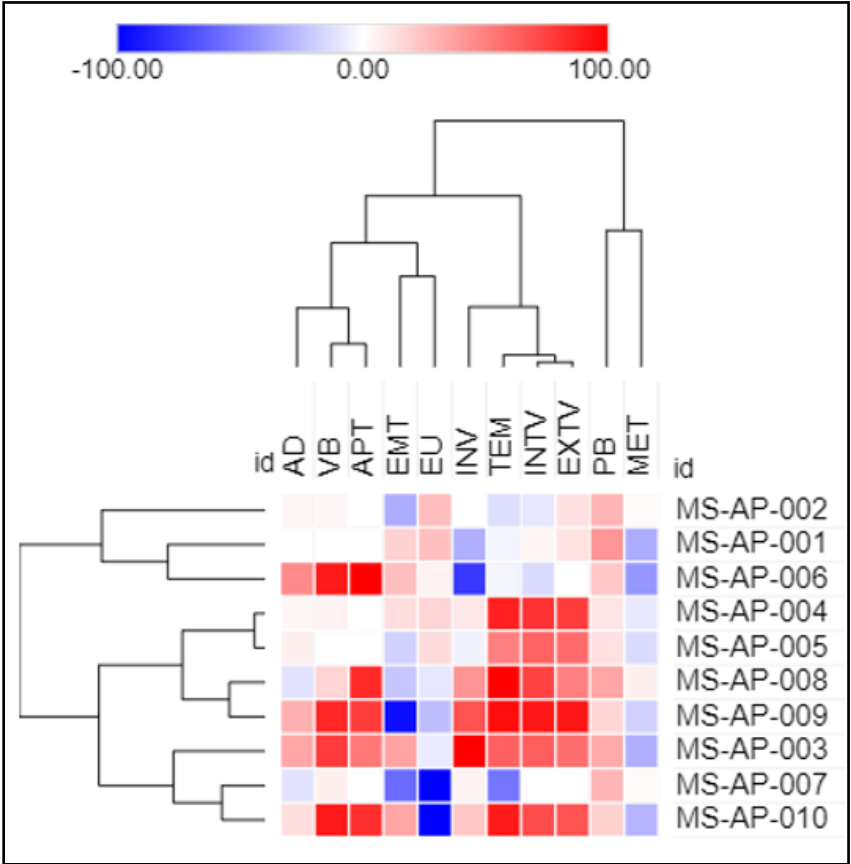
Non-cancer medications that has effect on survival



Effect of non cancer medicines on survival / death



Clinical data based weightage



METAssay data

Summary

- Machine learning algorithm, defining weightages, passed QC as it identified the correct parameters responsible for survival/death and rank-ordered them.
- Using the same program, other drugs were identified for their effect on survival (both positive and negative correlation)
- MS-AP-008 seems relevant in clinical settings in positively correlating with survival.
- MS-AP-008 clinical data matches with METAssay and METVivo data.
- Weaker compounds from the METAssay heat map are shown to correlate negatively with survival in the clinical setting, e.g MS-AP-001 and 005.
- Rank order of MS-AP-001 and MS-AP-005 from the METAssay panel also matches in the clinical setting.

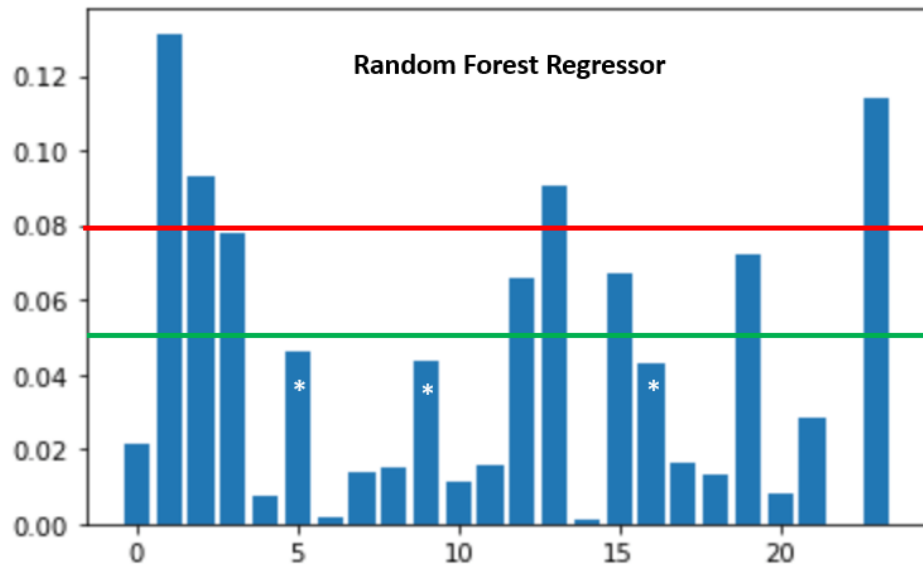
METAssay™ & METVivo™ can triage compounds that are clinically relevant

CASE STUDY II – Identification & Validation of Novel target

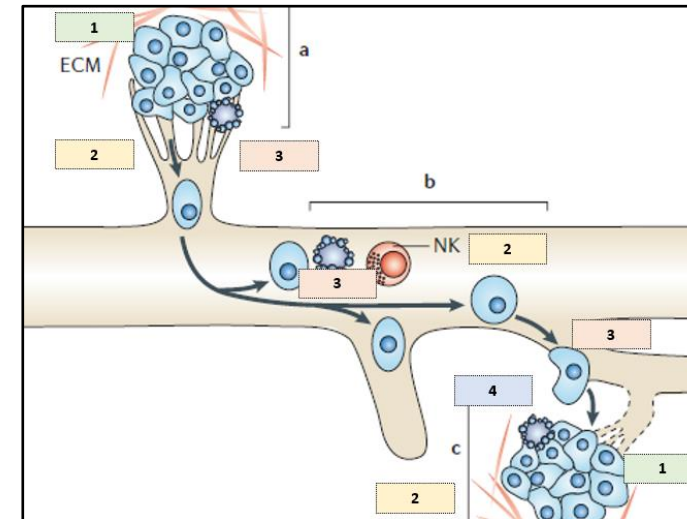
METAssay™ to METSCAN™: Identifying targets

METAssay™ wet lab data from cell lines and patient samples

Identification of 4 novel targets, all first-in-class for metastasis



METSCAN™ Identifies weighted rate-limiting steps

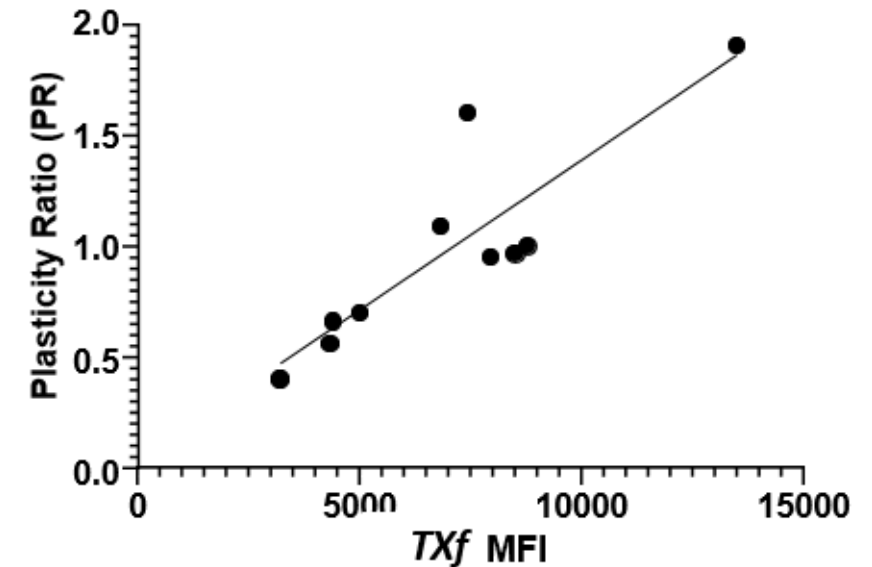


Identification of four novel first-in-class targets for metastasis

Case Study: Targeted Discovery PoC established



1. *TXf* OE and CRISPR in colorectal cancer
2. *TXf* OE and CRISPR in triple-negative cancer
3. Pharmacological intervention of weighted steps both in 2D & 3D
4. Pharmacological intervention in patient tumour samples
5. Biochemical assay standardized

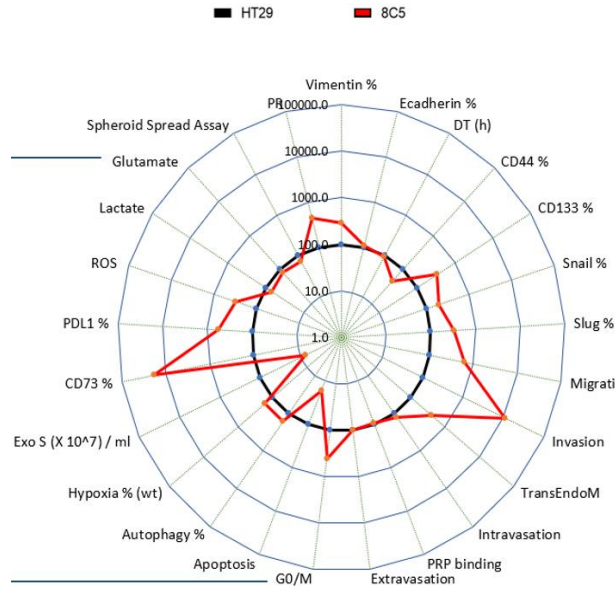


Positioned to start the first drug discovery programme

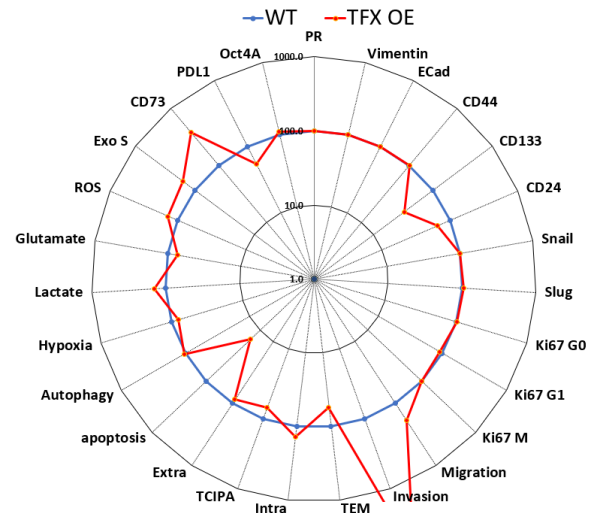
Discovery: genetic validation of first-in-class target

Overexpression

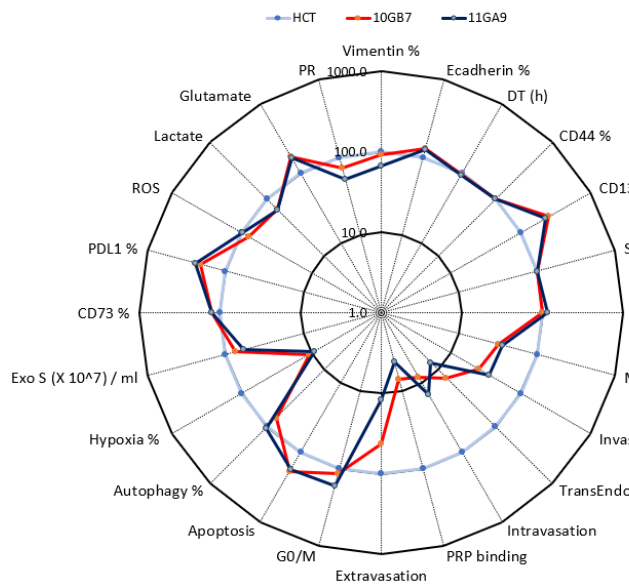
CRISPR CAS



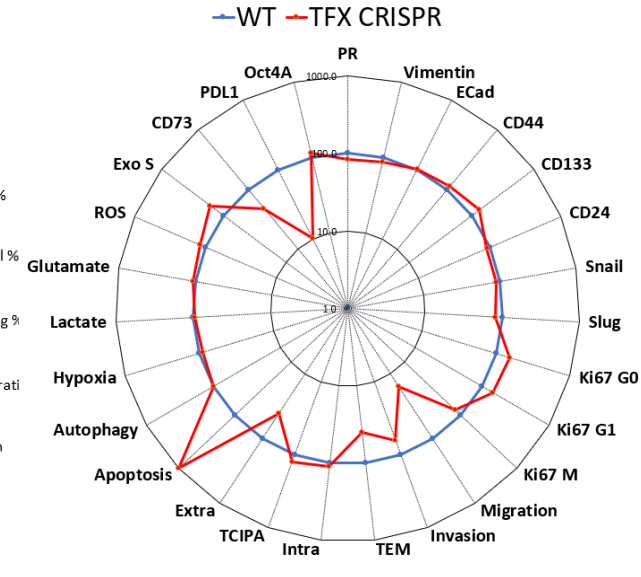
Colon



TNBC



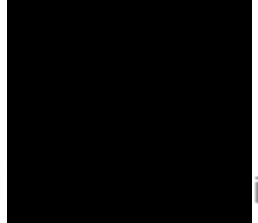
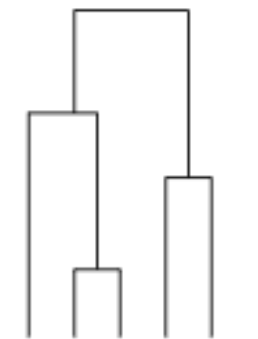
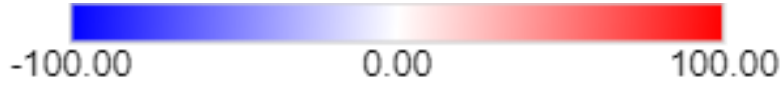
Colon



TNBC

Target Validation: Pan tumor effect * – both in 2D & 3D

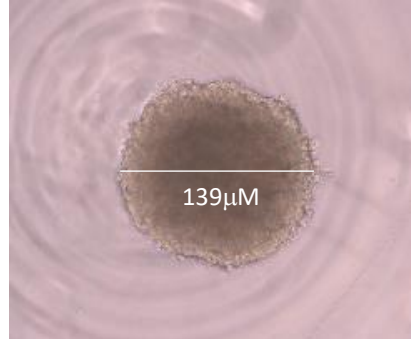
Percent Inhibition (Tool C)



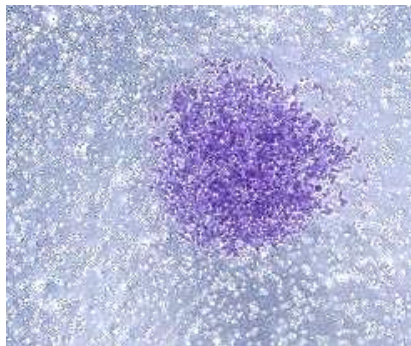
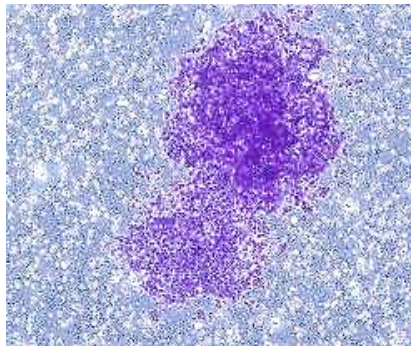
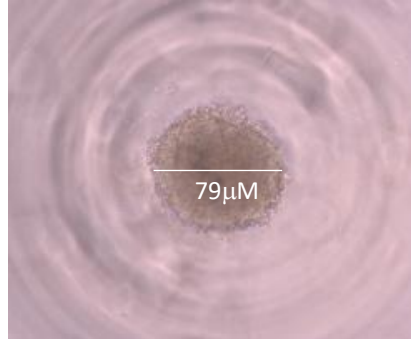
id	id
[Red]	HCT 116
[Red]	MDA MB 231
[Red]	Colo 205
[Blue]	HT29#8C5
[Blue]	HT29#12BC6
[Red]	MDA MB 468

*Independent of p53

HCT116 DMSO

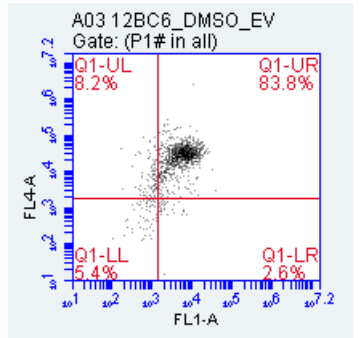


HCT116 + Tool C

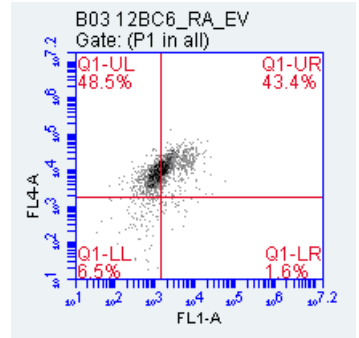


Cell line confirmation

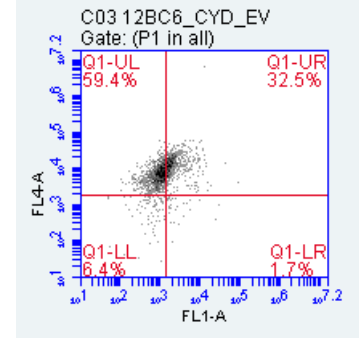
Control
PR = 0.97



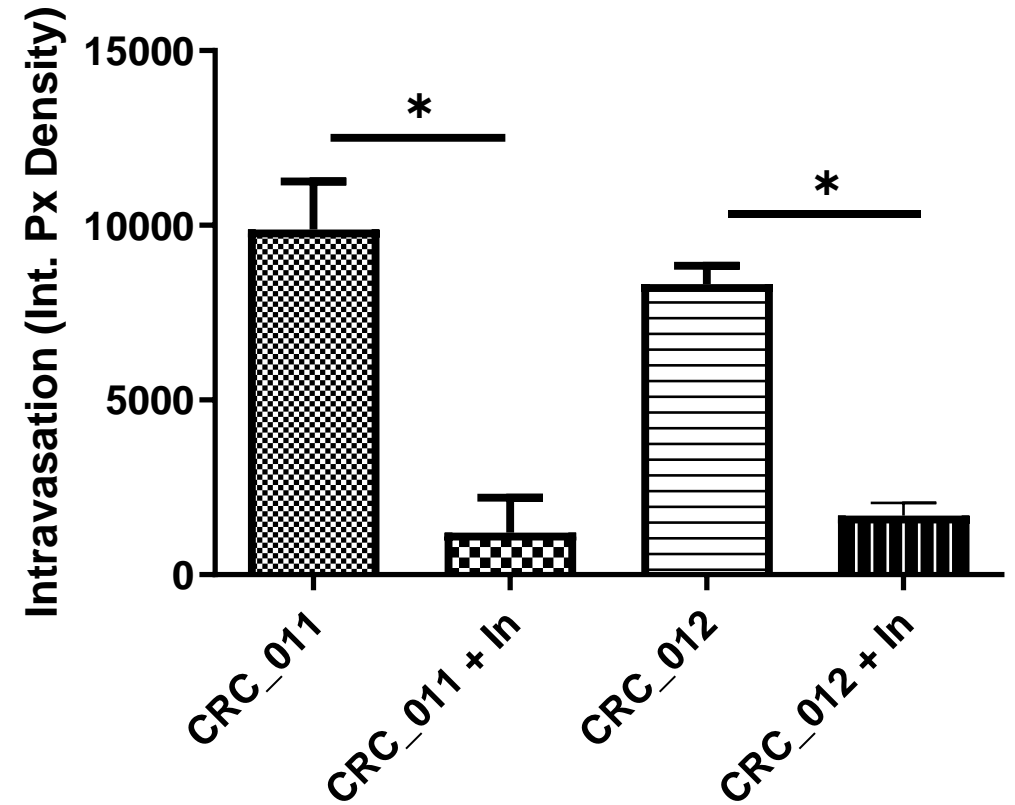
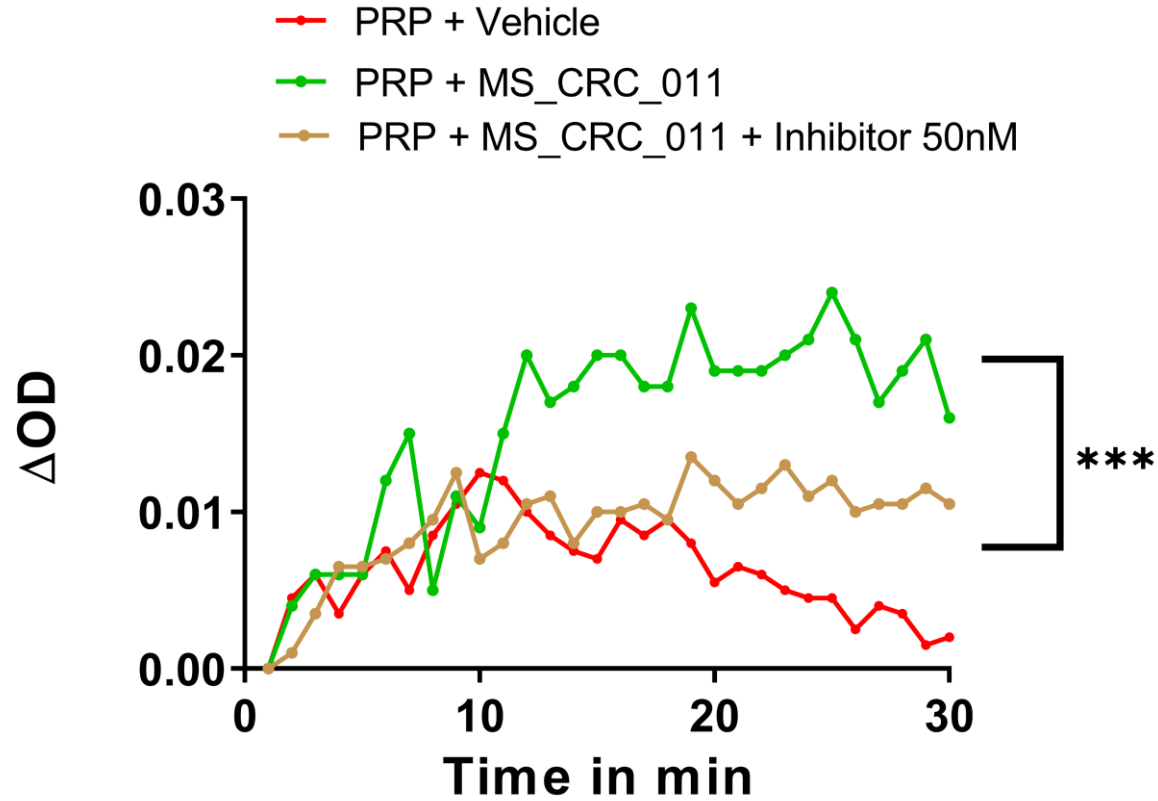
+ ve Control
PR = 0.54



Tool C
PR = 0.42



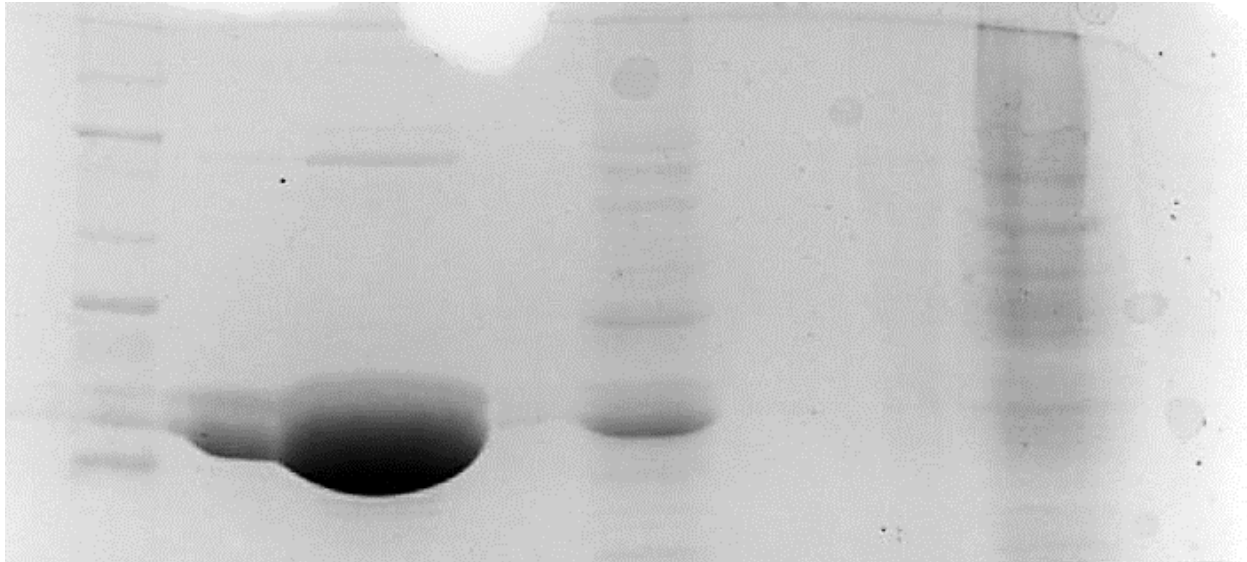
Target Validation: In Patient Samples



Patient confirmation

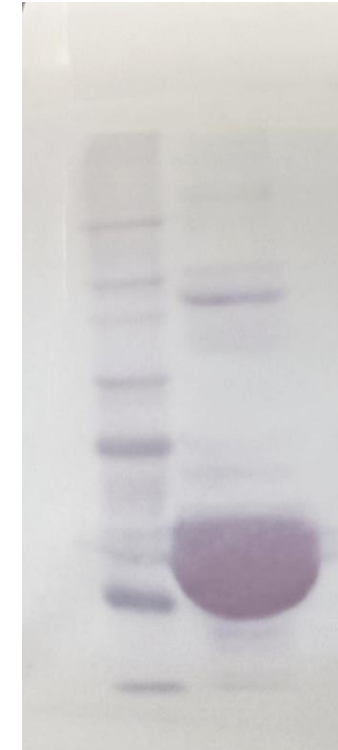
Expression & purification of *TXf*

Marker Elute Flow Through Load



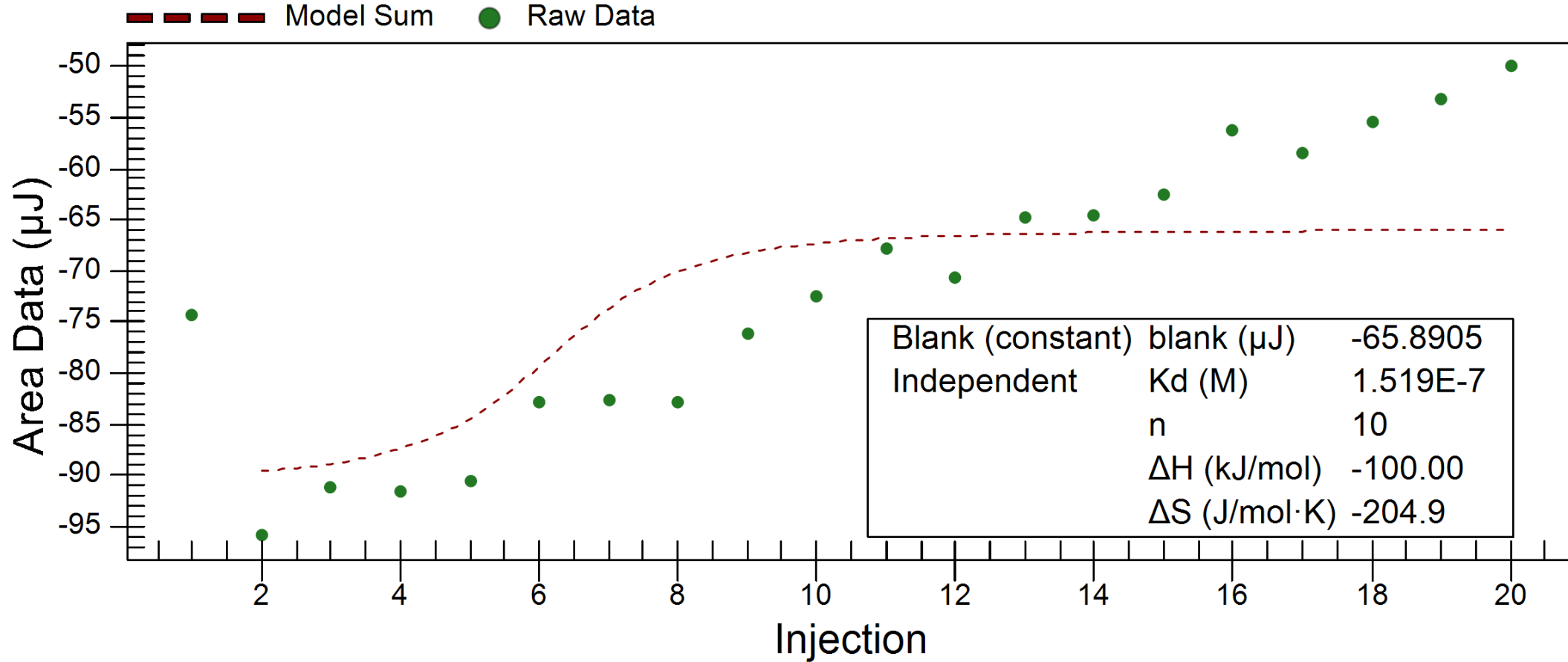
Gel Electrophoresis

Marker Elute



Western Blot

Biochemical assay optimization - ITC



Summary

- Algorithm identified eight critical steps
- Four first-in-class targets identified that play a critical role in these steps, especially in the latter part of the metastasis biology
- One target was the same TXf that was used for genetic engineering
- TXf purified and biochemical assay standardised

METSCAN™ can identify clinically relevant rate-limiting steps of metastasis

CASE STUDY III – Companion Diagnostics

METSCAN™ : Prediction of metastasis probability

Machine learning, prediction of metastatic probability

Goal: Predict the metastatic probability of treatment-naive primary tumours,

M0 (no metastasis), Any tumour grade or node status (blinded)

Support Vector
Machine
(92.3% Accuracy)

The first trial completed in CRC (13 patients)– no false negatives, three matches to date

A second trial ongoing for both CRC & H&N

International
Patent filed



METSCAN™, a novel and proprietary algorithm to predict the metastatic potential of primary tumor patients

MRS METASTASIS RESEARCH SOCIETY

Tamir Singh¹, Anshu Mishra¹, Sumit Roy¹, Debajyoti Roy Choudhury¹, Jati Tyagi¹, Anurag Mittal¹, Dinku Dora¹ and Anshu Roy Choudhury¹
¹Metastasis Inc, Madhav Vihar and ²Metastasis Solutions, Bangalore, India, ³Rajiv Gandhi Institute of Cancer Research, Delhi, India

INTRODUCTION

- The probability of primary colorectal tumor patients developing metastases is currently dependent on their node status, which is not always accurate.
- Metastasis has integrated the functional properties from primary tumor tumor-derived cells into a learning algorithm to predict the metastatic potential of pathological non-metastatic grade patients, blinded of tumor staging or node status.
- We have duplicated the complex and complex metastasis biology as data-distinguishing functional differences between metastatic and primary tumor cells in multiple cell lines, represented by the METSCAN™ platform.
- Data derived from the platform was used to train a learning algorithm METSCAN™ which distinguishes cells having different survival and growing phenotypes and semi-automatically predicts a tumor's probability to metastasize.

RESULTS

Transformed data fed into the machine learning model give an accuracy of 92.3%

- To further improve the accuracy and predicted test results, the most important features of cell line dataset were selected using the algorithm feature selection using five-to-eight and yielded an accuracy of ninety percent.
- To minimize false negatives and overfitting, the data set was further operational into a binary class label of metastatic and non-metastatic cells. Support-Vector-Machine showed 92.3 percent accuracy.

ML ALGORITHM	ACCURACY (%)
DECISION TREE	80%
SVM	80%
NAIVE BAYES	75%
LOGISTIC REGRESSION	65%

A Confusion Matrix of SVM Random Forest Regressor (on-off 0.85) of TRAINING DATA shows that:

- all MET labels has been predicted correctly and
- 1 non-MET label has been predicted as MET.
- Receiver operating characteristic curve (ROC) & Precision recall (AUPRC) was used for quality check.

Sample ID	Original Stage	Metastatic Potential	Accuracy	Metastatic Prediction	Percent Accuracy
CRC-001	M0	pT4N2b	0.00	Non-MET	0.00%
CRC-002	M0	pT2N1	0.00	Non-MET	0.00%
CRC-003	M0	pT4N2b	0.00	Non-MET	0.00%
CRC-004	M0	pT2N1	0.00	Non-MET	0.00%
CRC-005	M0	pT2N1	0.00	Non-MET	0.00%
CRC-006	M0	pT2N1	0.00	Non-MET	0.00%
CRC-007	M0	pT2N1	0.00	Non-MET	0.00%
CRC-008	M0	pT2N1	0.00	Non-MET	0.00%
CRC-009	M0	pT2N1	0.00	Non-MET	0.00%
CRC-010	M0	pT2N1	0.00	Non-MET	0.00%
CRC-011	M0	pT2N1	0.00	Non-MET	0.00%
CRC-012	M0	pT2N1	0.00	Non-MET	0.00%
CRC-013	M0	pT2N1	0.00	Non-MET	0.00%
CRC-014	M0	pT2N1	0.00	Non-MET	0.00%
CRC-015	M0	pT2N1	0.00	Non-MET	0.00%
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CRC-018	M0	pT2N1	0.00	Non-MET	0.00%
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CRC-024	M0	pT2N1	0.00	Non-MET	0.00%
CRC-025	M0	pT2N1	0.00	Non-MET	0.00%
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CRC-037	M0	pT2N1	0.00	Non-MET	0.00%
CRC-038	M0	pT2N1	0.00	Non-MET	0.00%
CRC-039	M0	pT2N1	0.00	Non-MET	0.00%
CRC-040	M0	pT2N1	0.00	Non-MET	0.00%
CRC-041	M0	pT2N1	0.00	Non-MET	0.00%
CRC-042	M0	pT2N1	0.00	Non-MET	0.00%
CRC-043	M0	pT2N1	0.00	Non-MET	0.00%
CRC-044	M0	pT2N1	0.00	Non-MET	0.00%
CRC-045	M0	pT2N1	0.00	Non-MET	0.00%
CRC-046	M0	pT2N1	0.00	Non-MET	0.00%
CRC-047	M0	pT2N1	0.00	Non-MET	0.00%
CRC-048	M0	pT2N1	0.00	Non-MET	0.00%
CRC-049	M0	pT2N1	0.00	Non-MET	0.00%
CRC-050	M0	pT2N1	0.00	Non-MET	0.00%

METHODS

CONCLUSION

- Among the blinded patient samples of the current blinded study, follow-up has identified three patients to be metastatic, all of which were correctly predicted by METSCAN™.
- There were no false negatives, but a few false positives, which may be indicative of higher platform sensitivity of METSCAN™.
- Further studies, with head and neck tumor patients are currently ongoing.
- The goal is to entrap: this platform for multiple epithelial carcinomas.

REFERENCES

Cancers, 2022, 14, 889 | Nature Reviews Clinical Oncology, 2019, 16, 187 | Nature Genetics, 2019, 51, 1113 | EACR, 2021, Poster P9-0134 | PCT applications # PCT/IN201059915 & PCT/IN202105928

MRS METASTASIS RESEARCH SOCIETY

13th-16th November 2022