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A Cell Based Phenotypic Assay Platform For Cancer Metastasis Drug Discovery And Diagnostics

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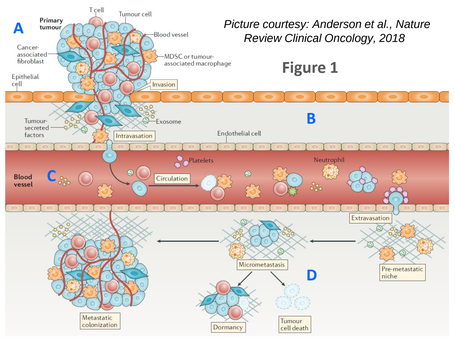
Introduction

1) Ninety percentage of cancer deaths are from metastasis, but there are very few approved treatments targeting the metastatic process[1]
2) Multiple late-stage failures in the clinic have led to de-prioritization of metastasis[2]
3) Drug discovery challenges plaguing metastasis are:
   a) Absence of translational assay platforms that successfully replicates metastasis biology on the bench
   b) Complete characterization of the specific moving cell population vis-à-vis metastasis biology is challenging
   c) Spontaneous animal models take a long time and are expensive
4) Colorectal cancer is 3rd most prevalent cancer globally (ten percent of all cancers) and is the fourth most common cause of death from cancer, estimated to be responsible for almost 700,000 cancer deaths[3].
5) The five-year survival rate is ninety percent for colorectal cancers diagnosed at an early stage compared with thirteen percent for those diagnosed at a late stage.

Methods

1) Break complex metastatic biology into simple, measurable cell-based phenotypic assays (Fig. 1 & Table 1)
2) Differentiate between growing and moving tumor cells with respect to their plasticity ratio (PR; the ratio of mesenchymal to epithelial markers)
3) Genetically engineer cells with high PR (Fig. 2 & 3)
4) Characterize the differential response of growing (cells with low PR) and moving cells (same cells but engineered, with high PR) on each of the cellular assays
5) Translational characterization of patient tumor samples & Identification of rate-limiting steps in metastasis



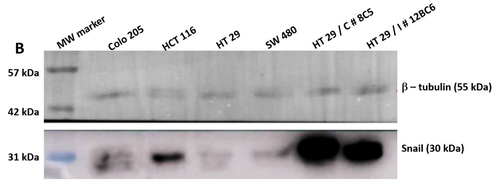
Picture courtesy: Anderson et al., Nature Review Clinical Oncology, 2018

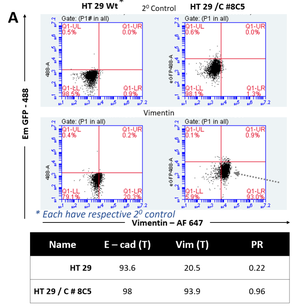
Figure 1

Results

Confirmation of engineered clones (post limiting dilution):

- Analysis of plasticity ratio (PR) (Fig. 3A)
- Expression analysis of pro-metastatic transcription factor Snail (Fig. 3B)
- Functional assay characterization (Fig. 3C)
- PR explains functional properties in various assays (Fig. 4) but NOT in ALL





Name	E – cad (T)	Vim (T)	PR
HT 29	93.6	20.5	0.22
HT 29 / C # 8C5	98	93.9	0.96

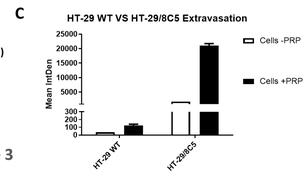
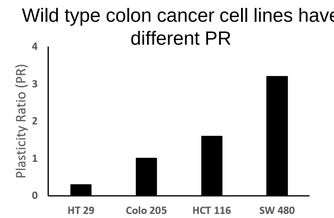
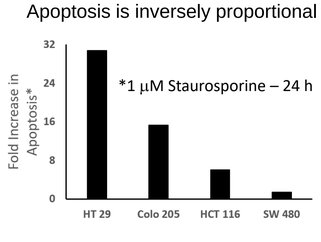
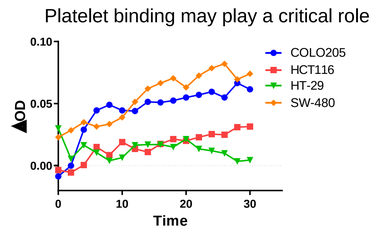


Figure 3







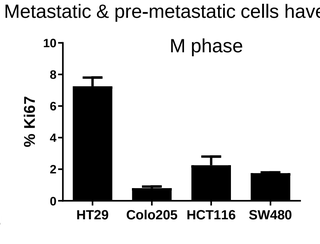
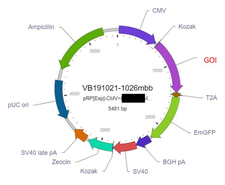


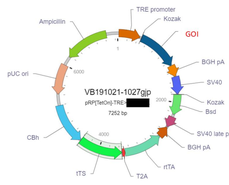
Figure 4

GROUPS	SL. NO.	MEASURED PARAMETER *(one marker / cellular assay per row, unless otherwise mentioned)*
CHARACTERIZATION (A)	1	Plasticity Ratio (PR; 2 markers)
	2	Eptihelial to mesenchymal transition (EMT)
	3	Stemness (2 markers)
	4	Doubling time
MOVEMENT - EPITHELIAL (B)	5	Adhesion
	6	Migration
	7	Invasion
MOVEMENT - BLOOD (C)	8	Transendothelial Migration
	9	Intravasation
	10	Surviving in blood
	11	Extravasation
SURVIVAL IN 2° TISSUE (D)	12	Mesenchymal to eptihelial transition (MET)
	13	Chemoresistance
	14	Survival Response (5 cellular assays)
	15	Cross Talking Ability (2 cellular assays)
	16	Immune Modulation (2 markers)

Table 1



Constitutive Vector



Inducible Vector

Figure 2

Petal Chart Analysis (log scale)

WILD TYPE COLON CANCER CELLS

HT 29 (WT AND METASTATIC, #8C5)

REPRESENTATIVE PATIENT SAMPLE

Figure 5 (non metastatic HT 29 normalized to base value of 100)

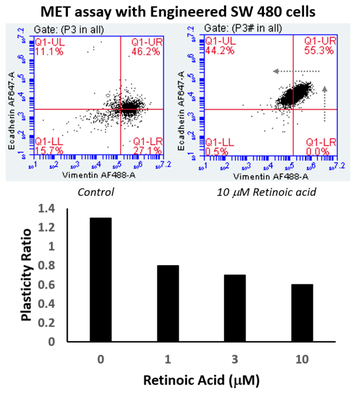
PR increase directly correlates with

CD44+CD133-
Trans endothelial migration
Extravasation
Autophagy
ROS
Exosome uptake
PDL1 expression

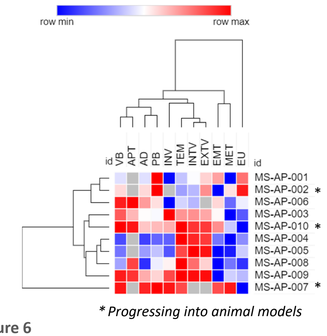
PR increase inversely correlates with

M phase
Apoptosis
Aerobic Glycolysis
Glutaminolysis
Exosome Secretion

Unique assay platform for drug discovery



- 10 approved drugs (selected by reading for on target effect in metastatic biology) across multiple indications
- Profiled across Mestastop assay panel
- One approved for metastatic colorectal cancer (009)



** Progressing into animal models*

Figure 6

Summary

- ✓ Mestastop has successfully created an in vitro phenotypic assay platform that summatively represent metastatic biology
- ✓ Characterization of wild type and proprietary engineered cells have identified PR as a critical determinant of metastasis and other functional properties of a tumor cell population

Way Forward

- ✓ Standardize proprietary in vivo model and generate PoC with identified approved drugs
- ✓ Assess 80 – 100 patient samples and identify the key rate-limiting steps for metastasis
- ✓ Drug discovery efforts around those steps

References

1. *Nature Reviews Clinical Oncology, 2019, 16, 185-204*
2. *European Journal of cancer, 2010, 46, 1177-80*
3. *https://www.wcrf.org/dietandcancer/cancer-trends/worldwide-cancer-data*

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Disclaimer

All patient studies are performed after approval of Ethics Committee and Institutional Review board of participating hospitals