Dissecting the 3D invasive properties of cancer cell lines and patient primary tumour cells and the impact of stromal cell co-culture

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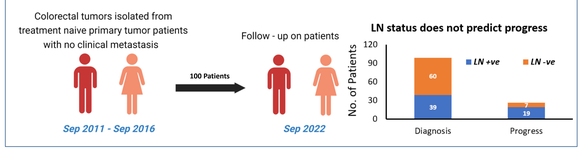
Background

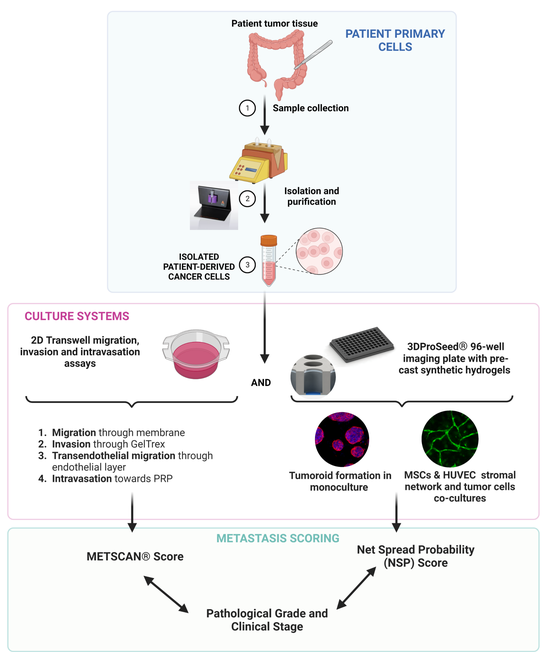
- All anti-metastasis drug discovery efforts targeting the invasion of cancer cells have failed in the clinic, suggesting the presence of other rate-limiting pathways[1].
- We have previously shown that multiple events in the colonization axis of disseminated cells (in the secondary tumour microenvironment) are critical for successful metastasis[2,3].
- Retrospective clinical trial data confirms that lymph node (LN) status does not predict metastasis probability (Fig 1).
- We have previously described a cellular, 2D phenotypic assay platform METSCAN® for predictive metastasis diagnostics, currently under clinical trials[4,5].
- Here, we discuss an alternate, shorter version of METSCAN® by assessing primary patient tumour cells in a 3D animal-free hydrogel model (3DProSeed®)[6] , with and without a stromal microenvironment.

Fig.1: Retrospective study in collaboration with Rajiv Gandhi Cancer Centre



Methods

Fig.2: Patient sample isolation and 2D/3D cell culture

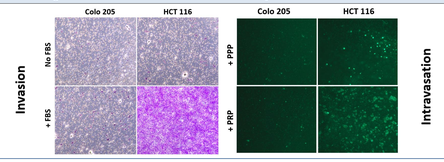


Results — Cell line based transwell assays

Table 1: Invasive properties DO NOT explain the metastatic probability

Cell Line	Migration	Invasion	TEM	Intravasation	Metastatic Source
HT 29	11.3	4.8	2.5	3.9	No
HCT116	659.6	166.3	16.9	11	No
SW480	***778.4***	***308.8***	***43.11***	***11.8***	***Yes***
Colo 205	***32.56***	***2.66***	***2.5***	***2.1***	***Yes***
HT29#12BC6	47.9	111.6	26.7	4.96	engineered
HT29#8C5	68.7	555.8	10.3	5.1	engineered
BT549	1071.8	982.5	24.6	258.3	Yes
MDA-MB-231	1077.2	962.3	6.4	9.5	Yes
MDA-MB-468	***389.1***	***427.9***	***4.5***	***17.1***	***Yes***
HCC 1937	***817.9***	***595.4***	***22.34***	***10.9***	***No***
CAL-27	1709.5	59	3.58	1.79	No
SCC-09	979	186	5.18	1.43	No
SCC-090	***0***	***0.001***	***1.97***	***1.29***	***No***
SCC-152	***502***	***50***	***3.47***	***1.56***	***No***

Figure 4: Invasion & Intravasation of metastatic cells are less



Results — Patient sample based transwell assays

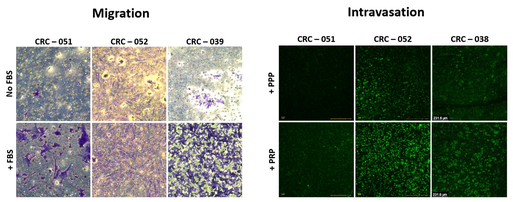
Table 2: Phenotypic properties DO NOT explain the metastatic probability

Patient ID	Migration	Invasion	TEM	Intravasation	TNM *(blinded)*
CRC – 037	288	26.8	2.3	4.8	T3N1bM1
CRC – 038	65	8.2	5.5	3.6	T3N2bM0
CRC – 039	681	2700	6.5	4.9	T3N3bM0
CRC – 044	20	16.9	3.51	42.5	T3N1bM0
CRC – 051	1579	5936	1.8	2.5	T4N2bM0
CRC – 052	253.8	389	3.9	22.4	T3N0M0
HNBM – 046	24.2	5.5	4.3	4.9	T3N0M0
HNBM – 050	64	13.2	7.9	13.7	T4aN0M0

**Fold increase, compared to the absence of chemoattractant*

- CRC-037 is from a primary tumour that has already metastasized but shows lesser invasive properties than CRC-039, isolated from a primary tumour yet to metastasize.
- The lymph node-negative CRC-052 shows higher migration and invasion than lymph-positive CRC-038 and CRC-044

Figure 5: Representative pictures from Table 2



Results — Cell line-based 3D monolayer culture

Figure 6: Cell line 3D mono culture – Baseline Creation for Invasion

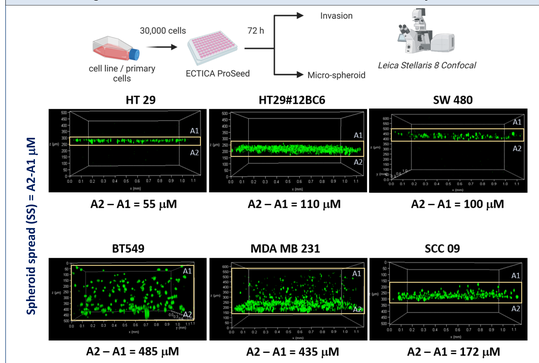
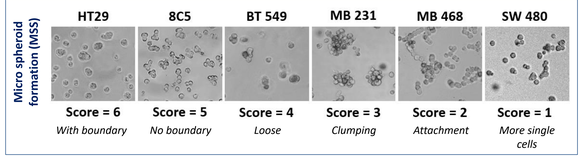


Figure 7: Cell line 3D mono culture – Baseline Creation for Micro-spheroid Formation



Results — Patient cells 3D monolayer culture

Figure 8: Patient tumour 3D mono culture – Invasion & Micro-spheroid Formation (CRC)

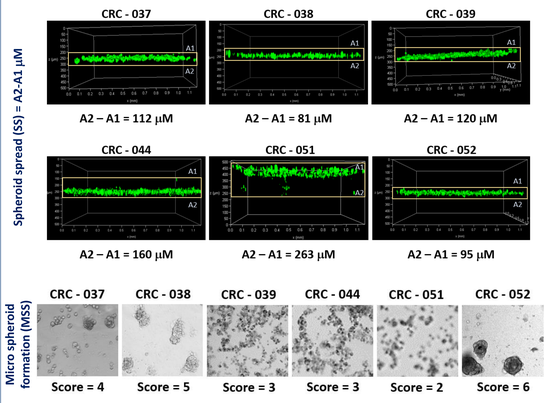
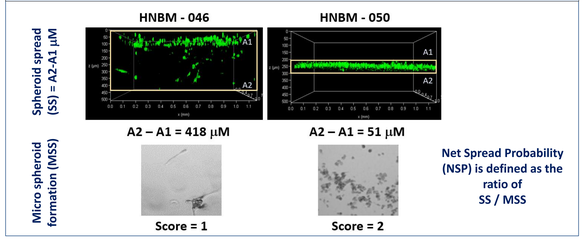


Figure 9: Patient tumour 3D mono culture – Invasion & Micro-spheroid Formation (HNC)



Results — Prediction of spread probability

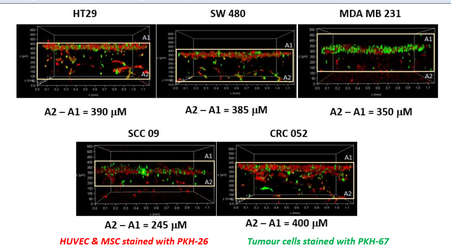
Table 3: Calculation of Net Spread Probability (NSP)

Patient ID	Spread (µM) (S)	Micro-spheroid score (MSS)	Net Spread Probability (NSP)	Pathological Grade *(blinded)*	Clinical Staging *(blinded)*	METSCAN® Score
CRC-037	112	4	28	pT3N1b	M1	0.65
CRC-038	81	5	16.2	pT3N2b	M0	0.7
CRC-039	120	3	40	pT3N3b	M0	0.99
CRC-044	160	3	53.3	pT3N1b	M0	0.76
CRC-051	**263**	**2**	**131.5**	**pT4N2b**	**M0**	**1**
CRC-052	**95**	**6**	**15.8**	**pT3N0**	**M0**	**0.39**
HNBM-046	418	1	418	pT3N0	M0	0.57
HNBM-050	102	2	51	pT4N0	M0	0.65

METSCAN® Score: a) Non-Met: 0.5 <; b) Pre – Met: >0.5-0.8<; c) Met: >0.8

- Net Spread Probability is a better predictive marker for metastasis than the invasion of tumoroids.
- But it is insufficient to explain the metastatic probability of all patients and would require further intervention.

Figure 10: Assessing the Impact of Stromal co-culture on 3D invasion



- MSC and HUVEC were added to the 3DProSeed®, on day 0 and day 3, respectively. Tumour cells were added on day 4 and cultured for another 72 hrs.
- Comparatively higher invasion was observed in the presence of tumour cells and stromal co-culture for most, including CRC-052.
- MDA-MB-231 showed lower invasion. The differential results needs to be evaluated and further characterized.

Summary

- Using patient tumoroids and 3DProSeed®, a 3D platform for predicting metastatic probability of primary tumours is being built.
- The platform currently distinguishes only the high metastatic cells from non-metastatic cells but not the intermediates.
- Further parameters and understanding the role of stromal cells needs to be incorporated to increase the efficiency.

References

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3. WO 2022/059026/A1 – Systems and methods for predicting cancer metastasis and screening of drugs
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