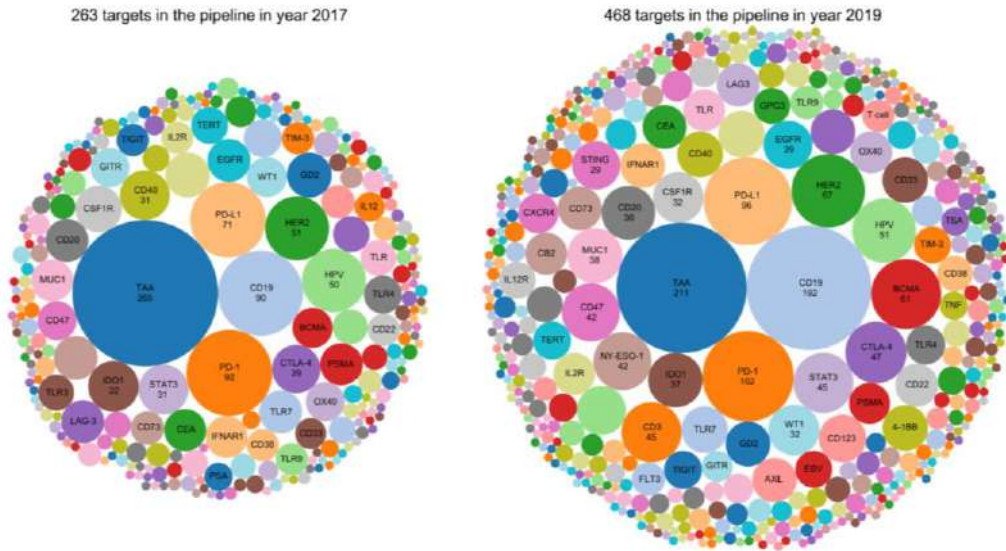


Botanicals in treating cancer:
How can molecular profiling and chemosensitivity help?



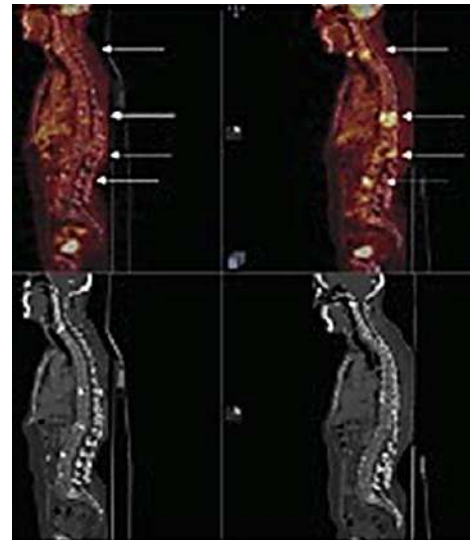
Clinical Needs

Increase of drug targets in oncology

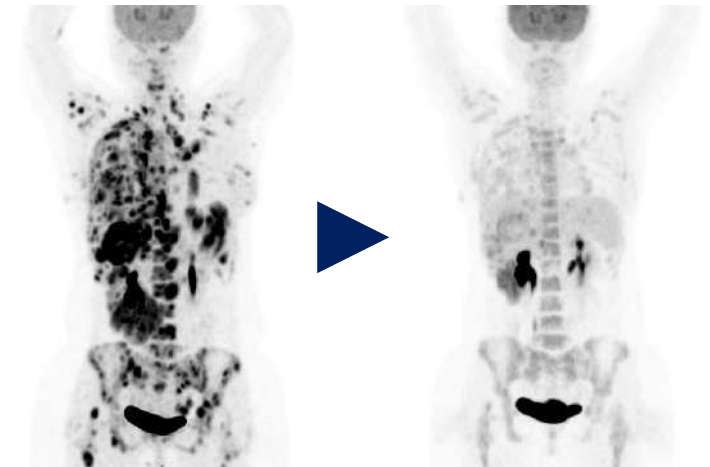


The increasing number of drugs is only sensible, when the diagnostic tools cover all targets

Advanced Salivary Duct Carcinoma



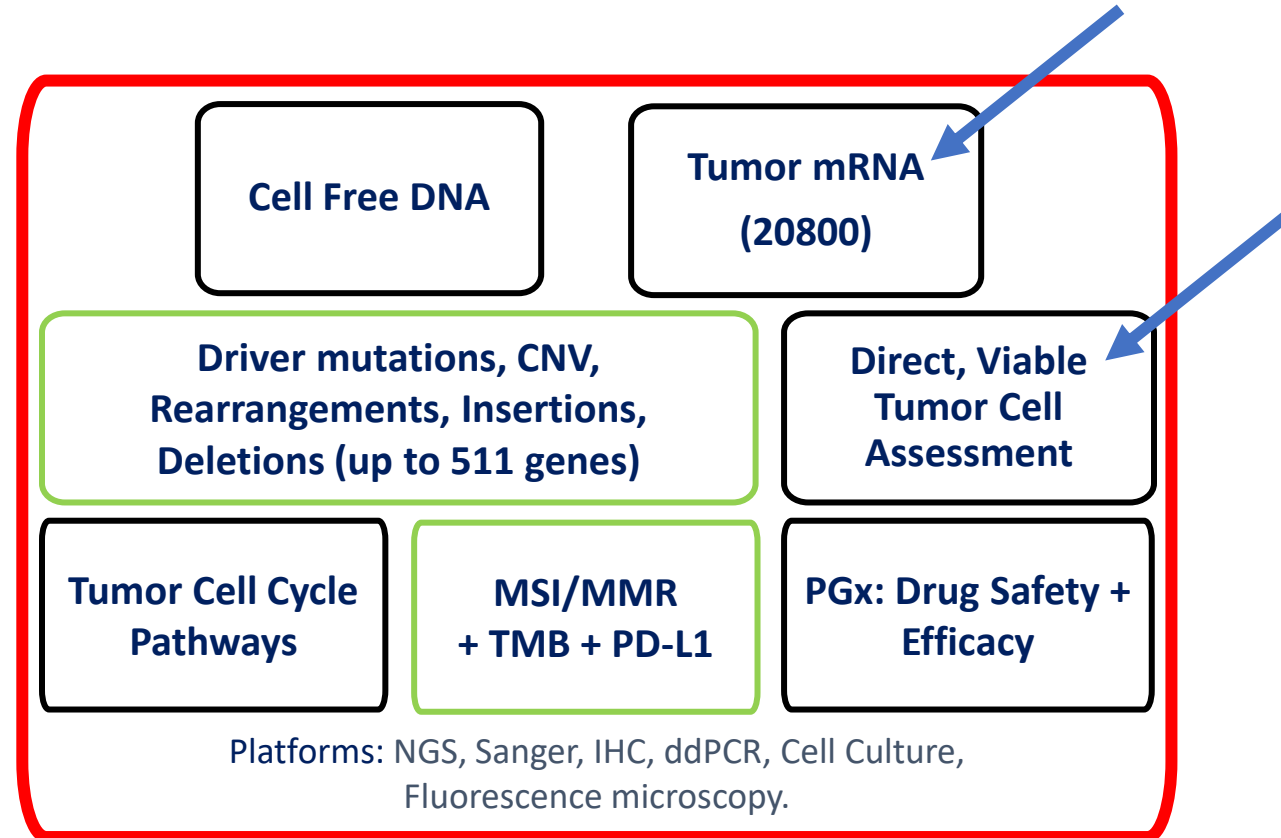
Stage IV Invasive Ductal Triple Negative Breast Cancer



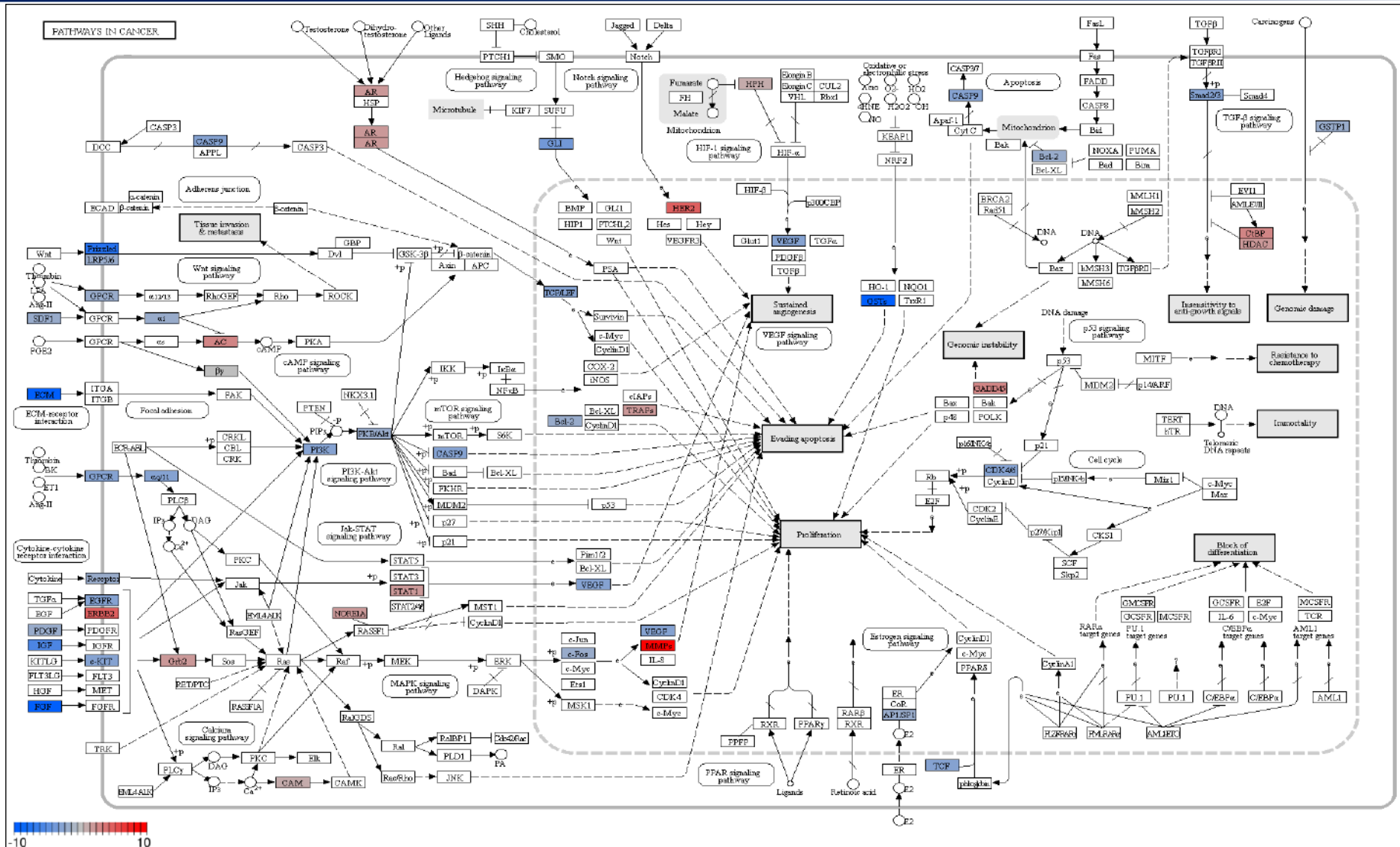
Cancer had progressed following 5 lines of therapy.

Administration of DCGL-recommended therapy led to regression.

Comprehensive Cancer Diagnostics

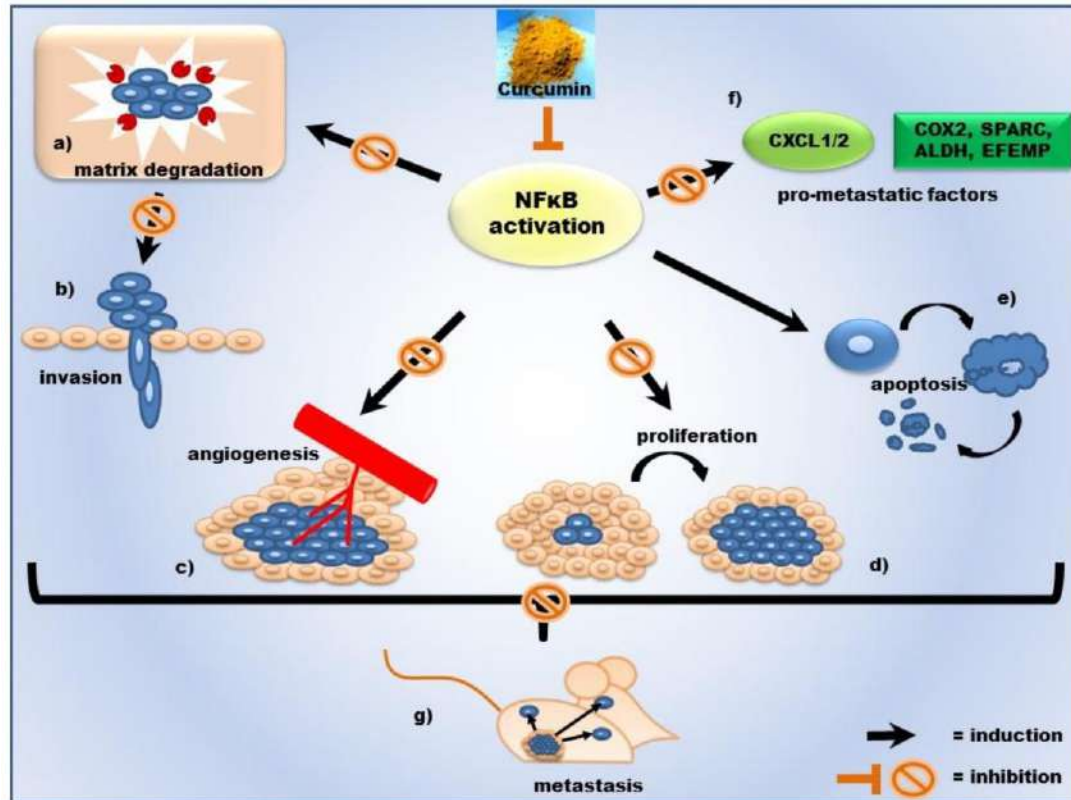


Important Pathways of Cancer



mRNA
quantification
from exosomes

Pathways relevant for Curcumin/Artesunate



Artesunate inhibits MMPs

Rasheed SA, Efferth T, Asangani IA, Allgayer H. First evidence that the antimalarial drug artesunate inhibits invasion and in vivo metastasis in lung cancer by targeting essential extracellular proteases. *Int J Cancer*. 2010 Sep 1;127(6):1475-85. doi: 10.1002/ijc.25315. PMID: 20232396.

Breast Cancer, female, 39

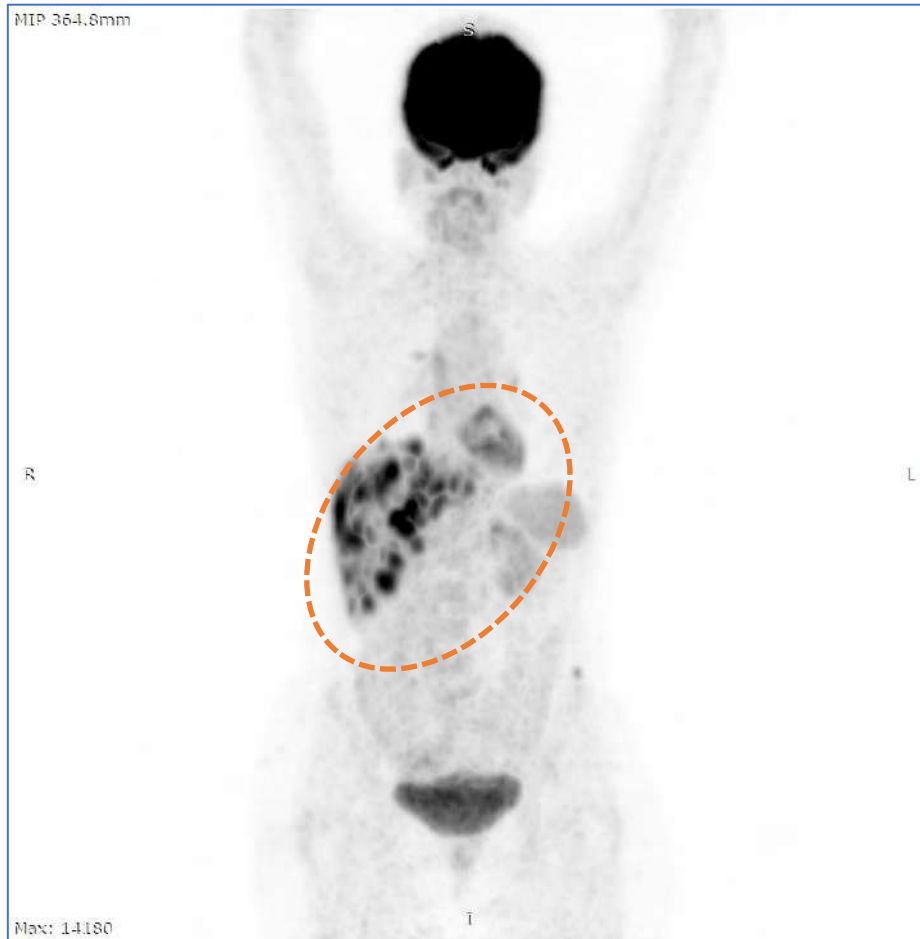
Clinical History

Date	Procedure
2015 (Jul)	R Breast fibroadenoma
2015 (Nov)	R Breast mass + lymphadenopathy
2016 (Jan)	Diagnosis on FNAC: IDC Grade III, ER+, PR-, Her2+
2016 (Mar)	Right MRM
2016 (Mar – Jun)	Doxorubicin + Cyclophosphamide
2016 (Jul)	EBRT (50 Gy, 25#) + Paclitaxel
2017 (Jan)	Progression to Stage IV: Lung + Liver metastases
2017 (Jan – Nov)	Trastuzumab + Paclitaxel + Maintenance Trastuzumab
2017 (Nov) – 2018 (Jan)	Gemcitabine + Cisplatin
2018 (Feb)	Progression: Increased liver metastases

Exacta Guided Therapy

Gene / Pathway / Analysis	Therapeutic Implication
PIK3CA mutation	Temsirolimus
VEGFA overexpression	Bevacizumab
MAPK activation	Atorvastatin
WNT Pathway activation	Quercetin
MMP activation	Doxycycline

Breast Cancer, female, 39



Medulloblastoma, male, 8

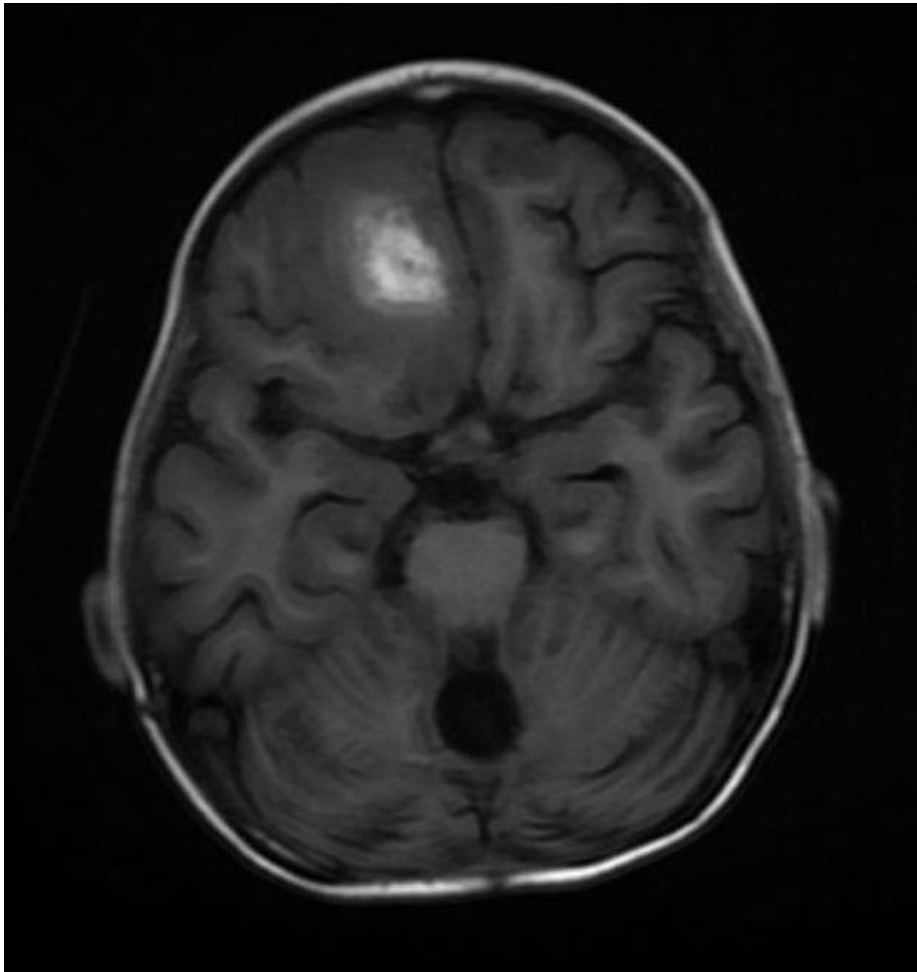
Clinical History

Date	Procedure
2016 (Oct)	Craniotomy + mass excision
2016 (Oct)	MRI: No residual disease
2016 (Dec)	New lesions in operative site + spinal metastases
2016 (Dec) – 2017 (Feb)	35Gy/21# + 20Gy/11# + 9Gy/5# to spine
2017 (Mar)	MRI: PR
2017 (Apr – Jul)	Earboplatin + 4-epiadriamycin + Teniposide
2017 (Aug)	NED
2017 (Sep) – 2018 (Jun)	COMBAT regimen (temozolomide + etoposide + celecoxib + vitamin D + fenofibrate + retinoic acid)
2018 (Jun)	MRI: New lesion in R frontal lobe

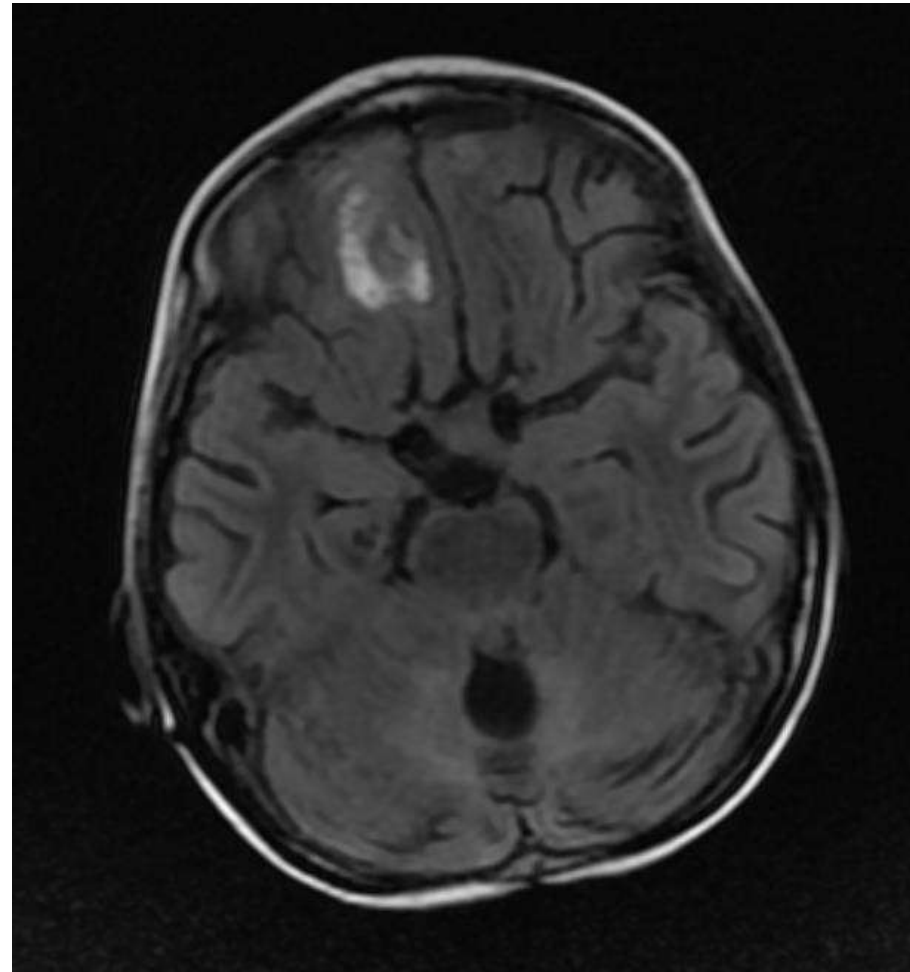
Exacta Guided Therapy

Gene / Pathway / Analysis	Therapeutic Implication
TOP2A expression	Doxorubicin
VEGFA Expression	Bevacizumab
ERBB2 Expression	Afatinib
Wnt Pathway	Quercetin
BIRC5 Expression	Curcumin

Medulloblastoma, male, 8

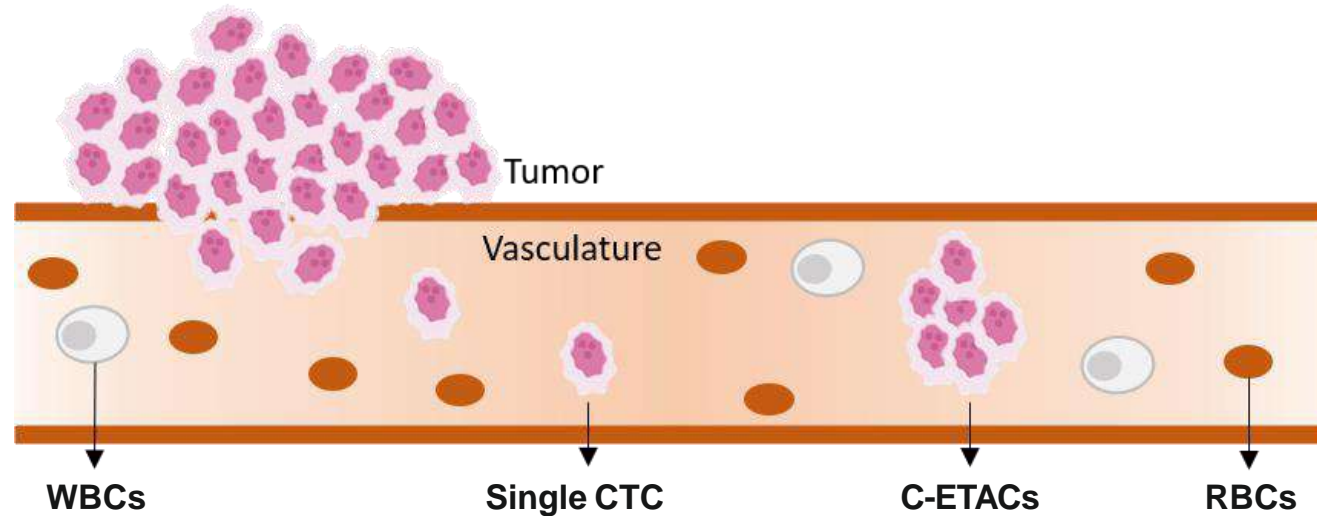


Jun 2018



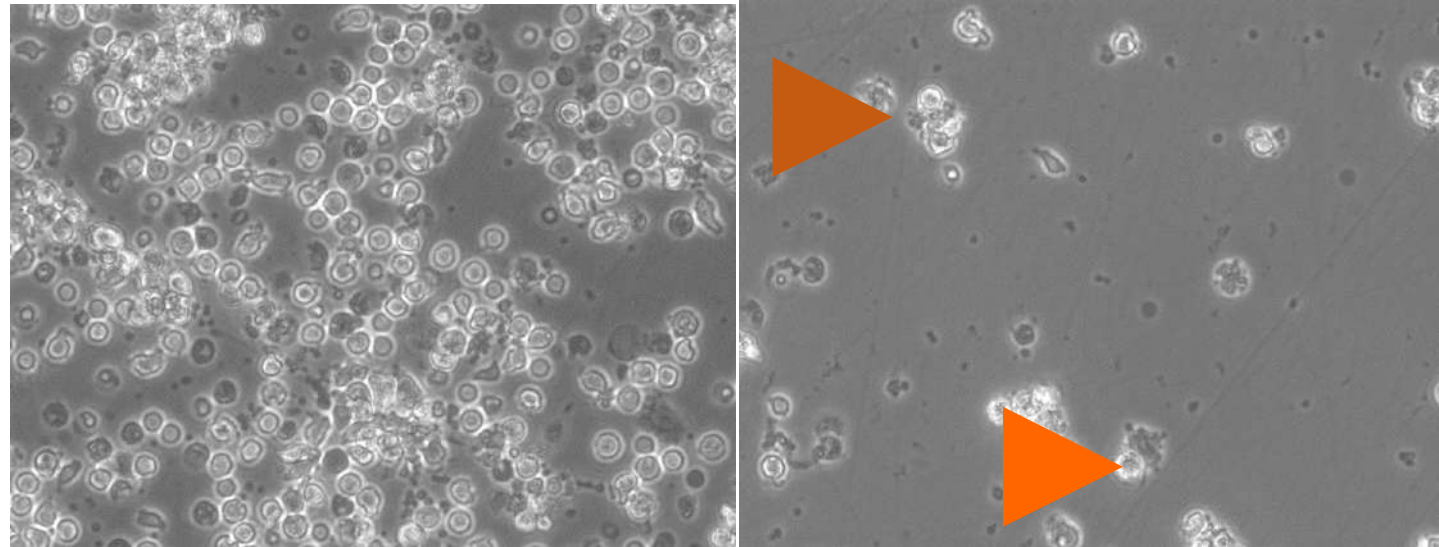
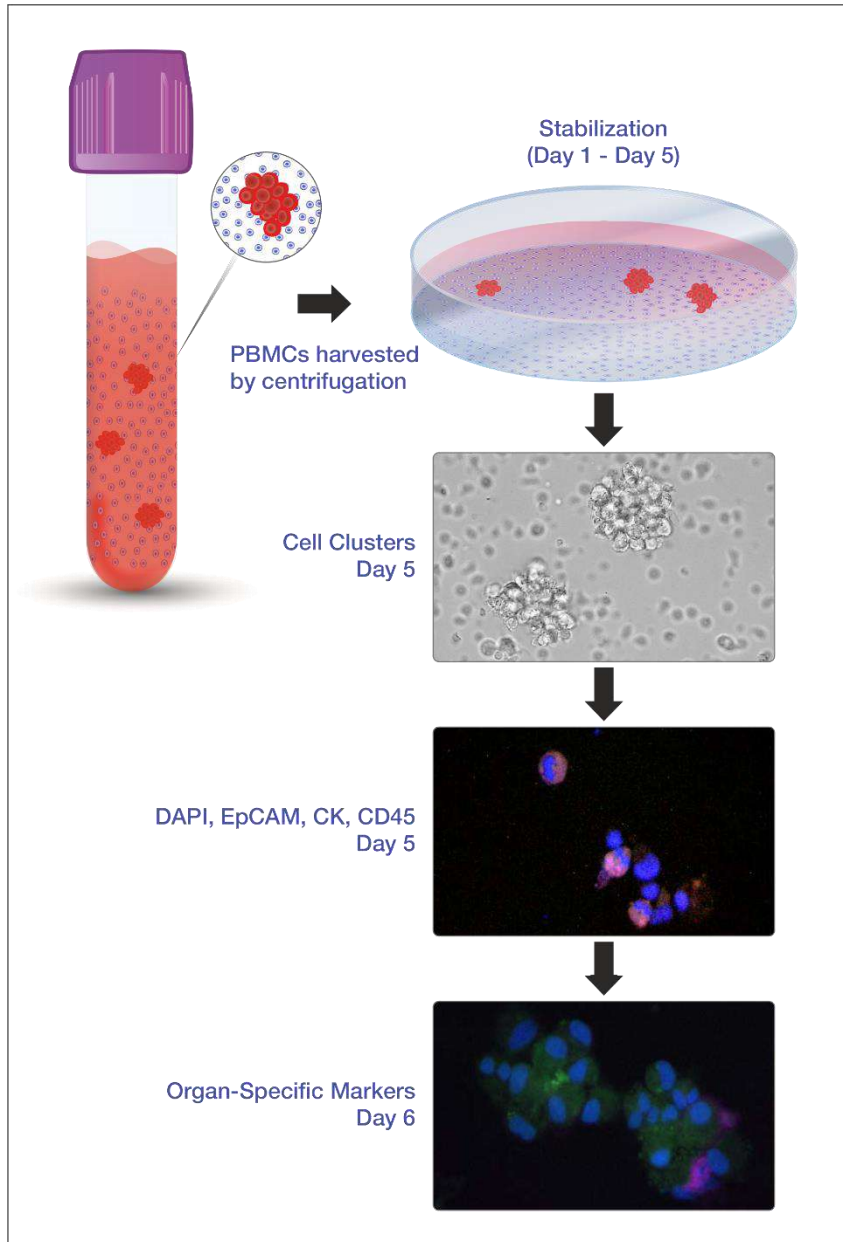
Jul 2018

Circulating (Ensembles of) Tumor (associated) Cells



- Single Circulating Tumor Cells (CTCs) and C-ETACs originate in the primary tumor or metastatic deposits.
- Visualization of C-ETACs offers direct visual evidence of the malignancy.
- C-ETACs are specific to the malignancy and absent in healthy individuals or in benign conditions.
- Detection of C-ETACs is not prone to interference from other factors such as inflammatory conditions.
- A sample of C-ETACs is equivalent to a micro-biopsy of the tumor

METHOD: Cell Wizard™



Day 1

Post Cell Wizard™

Cell Wizard uses medium with paradoxical cytotoxicity to kill cells with functional apoptotic mechanism while holding harmless apoptosis resistant cells and clusters in the PBMC milieu.

Any emboli / cluster like assemblages of cells surviving after 5 days of exposure to the special media were aspirated for characterization with immunofluorescent (IF) antibodies i.e. DAPI, EPCAM, CK and CD45.

(US Patent Office Application Numbers 62849840 and 62796098)



Certificate No.: MC-2309



How do we know our Method detects CTC?

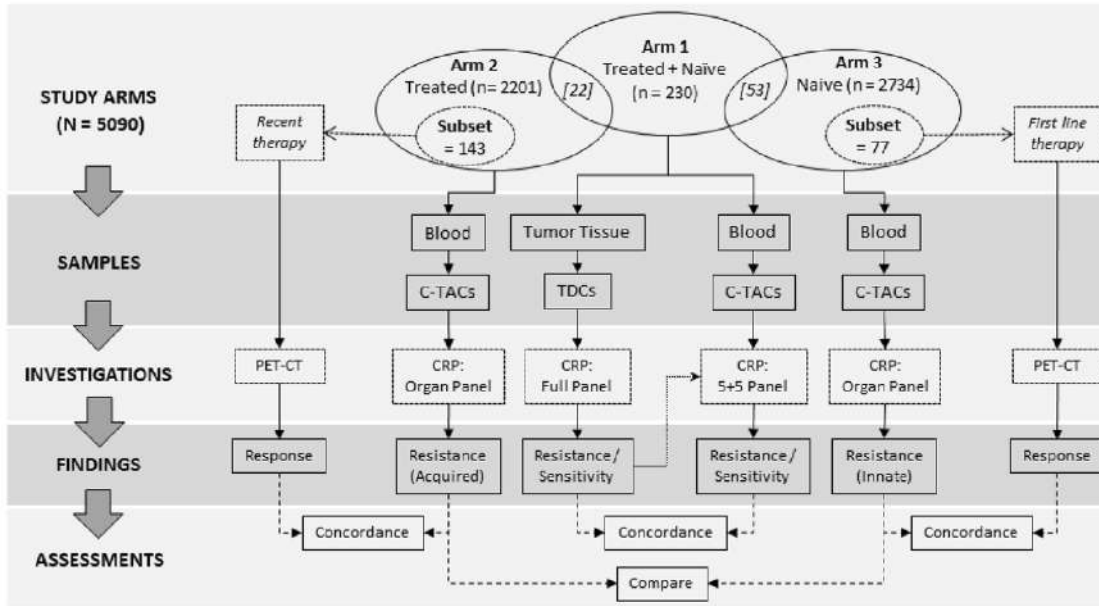
Breast Cancer Screening Trial Findings of ddPCR Assays

- Various gene variants detected in the tissue samples of 53 breast cancer cases by NGS.
- ddPCR analysis of these variants in genomic DNA isolated from CTCs, showed an overall 81.1% concordance with findings on tumor tissue.
- Similar Findings in Prostate Cancer.

Target Assay	Gene ID	Control Plasmids	Samples		
			Total	Positives	Negatives
AKT1_E17K	AHWSLXQ	34R2	7	5	2
EGFR_D855N	AHFBBKR		2	2	0
ESR1_Y537N	AHCTE56		3	3	0
GNAS_R201C	AH6R7EO	24R3	1	1	0
GNAS_R201H	AH705KW		3	2	1
KRAS_G12C	AHHS7X9		1	1	0
PIK3CA_E545Q	AH21CV0		1	0	1
PIK3CA_E542K	AHKA4AQ	24R5	7	5	2
PIK3CA_E545K	AHLJ2GY		8	6	2
PIK3CA_H1047L	AHPAWZM	24R5	1	1	0
PIK3CA_H1047R	AHD2DCF		9	8	1
PIK3CA_N345K	AHHS7YA		2	2	0
TP53_R248Q	AHVJNUO	24R3	3	3	0
TP53_R248W	AHRSTB0		1	0	1
TP53_R249S	AHX1J64		1	1	0
TP53_R273H	AHUAPOG		2	2	0
KRAS_G12V	AH0JGKY	Internal	1	1	0
OVERALL			53	43 (81.1%)	10

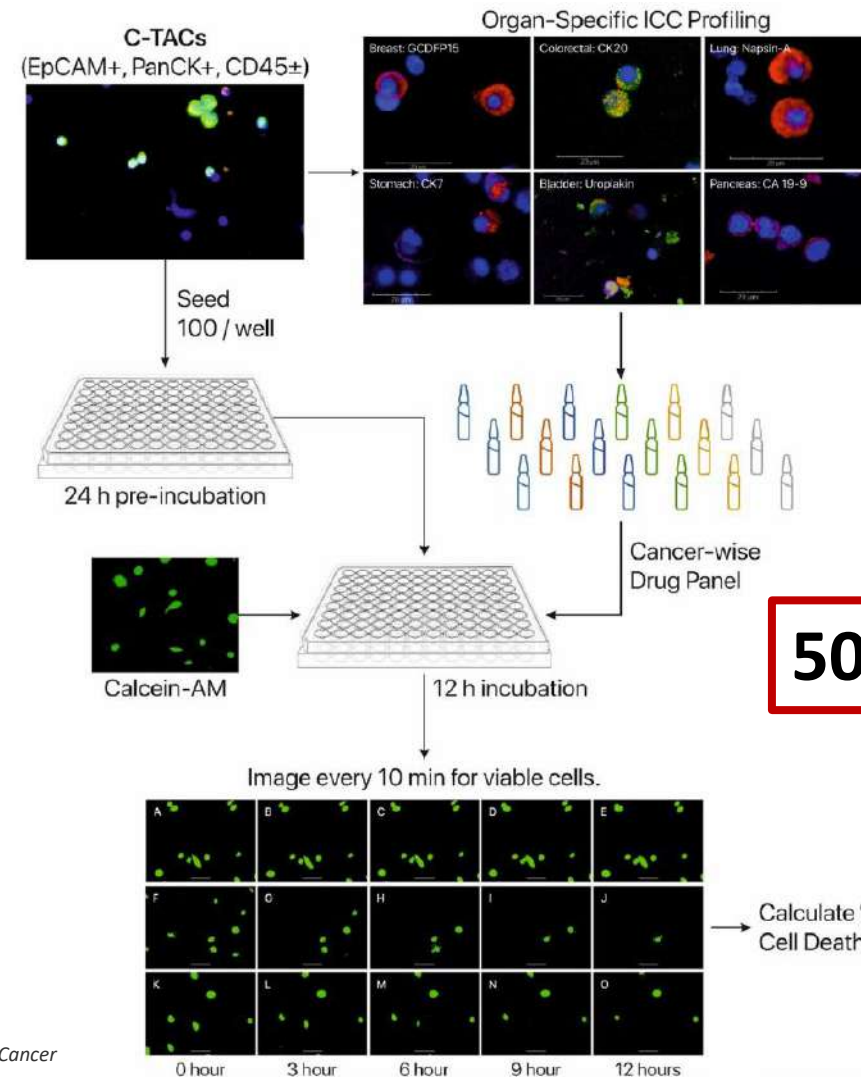
Crook, T.; Leonard, R.; Mokbel, K.; Thompson, A.; Michell, M.; Page, R.; Vaid, A.; Mehrotra, R.; Ranade, A.; Limaye, S.; Schuster, S., et al. Accurate Screening for Early-Stage Breast Cancer by Detection and Profiling of Circulating Tumor Cells. *Cancers* 2022, 14, 3341. <https://doi.org/10.3390/cancers14143341>

Therapy Management 2020 (Chemosensitivity Testing on CTACs)



**93,7%
Concordance
between tissue
and CTC**

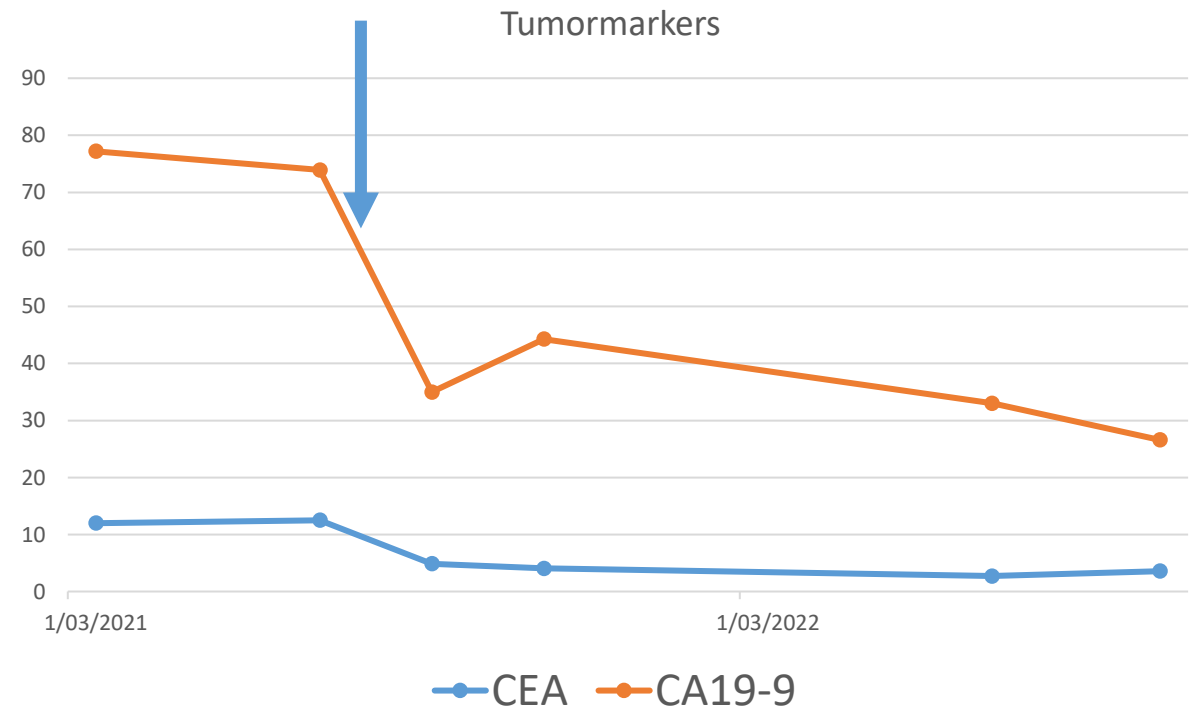
**In pretreated patients the
chemo profile of C-TACs
was in 87% concordant
with in vivo treatment
failure**



5000 Patients

Metastatic Mucinous adenocarcinoma of the appendix

- T4N2M1, male 49 years, 01.2021
- FOLFOXIRI, March 2021
- Stopped with Oxaliplatin June 2021
- Added i.v. Resveratrol+DMSO, and oral Melatonin and Quercetin since July 2021.
- Reduced 5FU, added Bevacizumab. October 2021
- In stable disease since 03/2021



Metastatic Colon Cancer, female, 42

- Diagnosed, 02/2020, T3N0M0 G2 Adenocarcinoma
- Surgery, 5-FU (Capecitabine)
- 08/2020 Metastasis lung
- 02/2021 Progression, Surgery,
- 08/2021 Comprehensive Tumor Testing, Artesunate/Vitamin C/Curcumin
- 11/2021 Nivolumab
- 12/2021 Liver Metastasis, Progression
- 01/2022 FOLFOX+Beva
- 08/2022 full remission

No	Drug	Indication	Dose/Schedule/Notes ²
1	5-Fluorouracil + Oxaliplatin + Bevacizumab	Chemosensitivity, VEGFA overexpression	Standard dose

DRUG	INDICATION
Curcumin	52% CD; MMP7 (+10.00 FC), MMP9 (+4.72 FC), MMP11 (+3.20 FC) overexpression
Apuxan	52% CD
Atorvastatin	52% CD; MAPK pathway activation - MAP3K10 (+2.70 FC) overexpression
Glibenclamide	52% CD
Hypericin	52% CD
Doxycycline	51% CD; MMP7 (+10.00 FC), MMP9 (+4.72 FC), MMP11 (+3.20 FC) overexpression
Aspirin	51% CD
Ascorbic Acid (Vitamin C)	49% CD; SLC2A1 (GLUT1) (+2.58 FC) overexpression
Artesunate	49% CD; MMP7 (+10.00 FC), MMP9 (+4.72 FC), MMP11 (+3.20 FC) overexpression
Cannabidiol	45% CD; MMP7 (+10.00 FC), MMP9 (+4.72 FC), MMP11 (+3.20 FC) overexpression

RESPONSE TO REPURPOSED DRUGS

Sr.No.	Drug/Drugs Combination	% Cell Death
1	Vitamin C	55
2	Cannabidiol	55
3	Apigenin	55
4	Indol-3-carbinol	54
5	Amygdalin	53
6	Doxycycline	51
7	Propranolol	49
8	Artesunate	49
9	Iscador P	48
10	Curcumin	47
11	Pascorbin	47
12	Hypericin	46
13	Apuxan	45
14	DMSO	45
15	Genistein	42
16	Dichloroacetate	41
17	Diflunisal	38
18	Helixor A	38
19	Helixor M	34
20	Helixor P	30
21	Melatonin	28
22	Resveratrol	26
23	Epigallocatechingallate	25
24	Aspirin	23
25	Quercetin	18
26	Iscador Qu	11
27	Glibenclamide	3
28	Glutathione	0

Response to Repurposed Drugs



Take Home Messages

- We can personalize complementary cancer treatment based on circulating tumor cells (C(E)TACS), Exosomes
- Alternative treatments can contribute to a successful treatment
- Limitations, especially for oral given substances (unclear pharmacokinetics)