Organoids for Drug Screening

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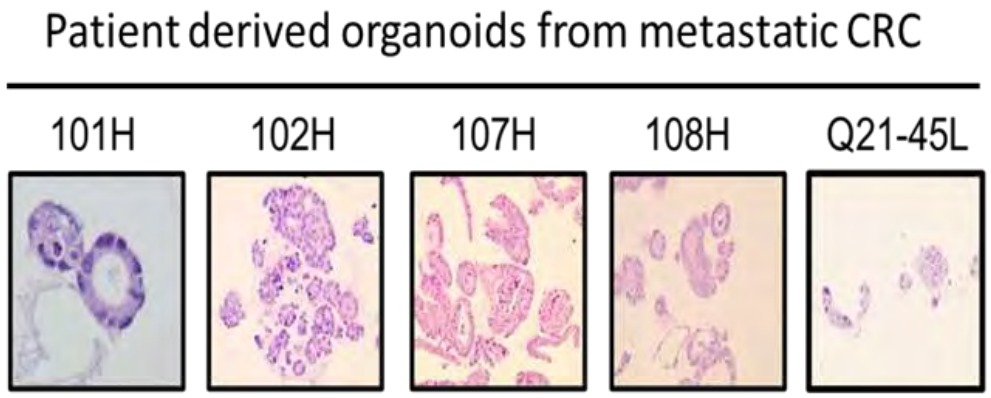
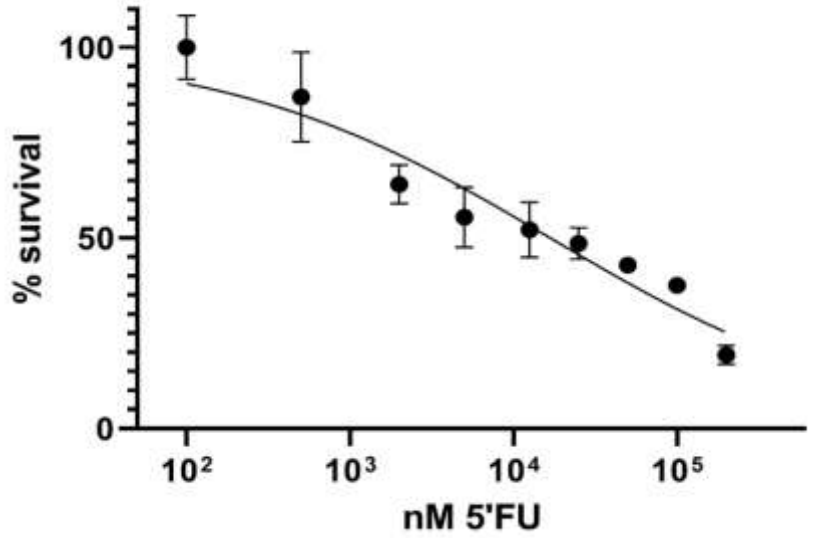




Objectives

1. Describe the most common culture technique for generation of patient-derived organoids.
2. List a few genetic mutations that have been shown in organoids to be targeted by novel therapies.
3. Describe some of the limitations of organoid culture.

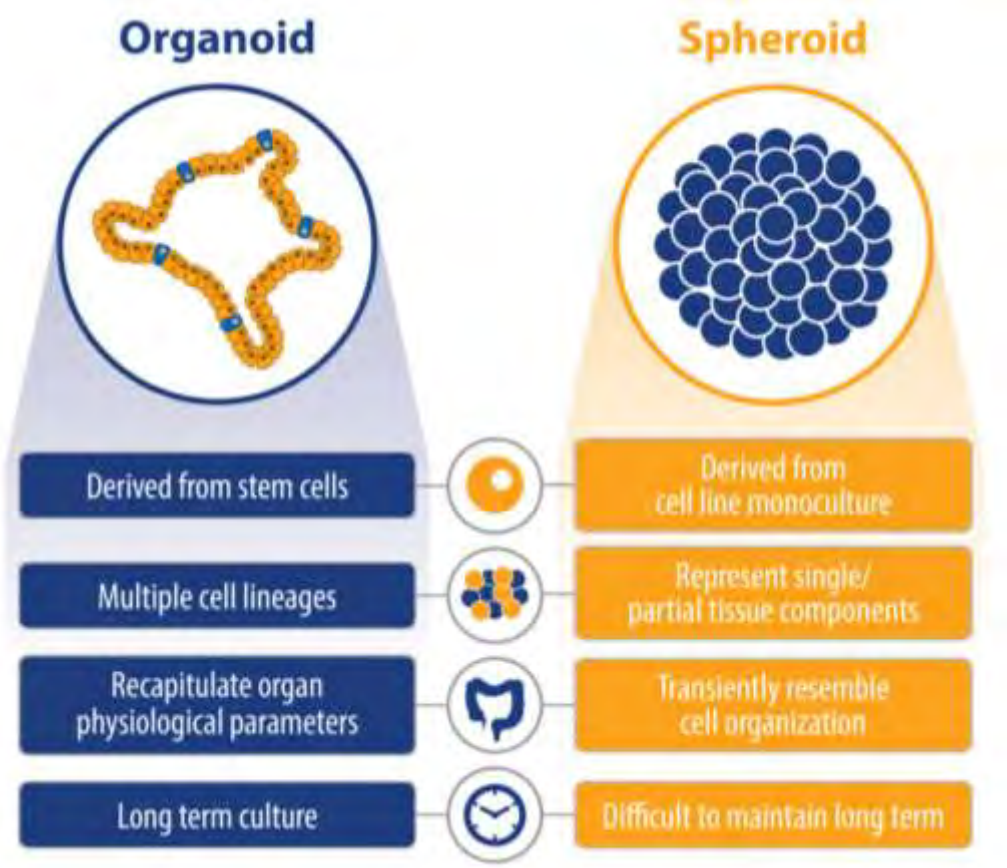
Disclosures: None

Patient tumors can give rise to cell lines, organoids, and xenograft cultures

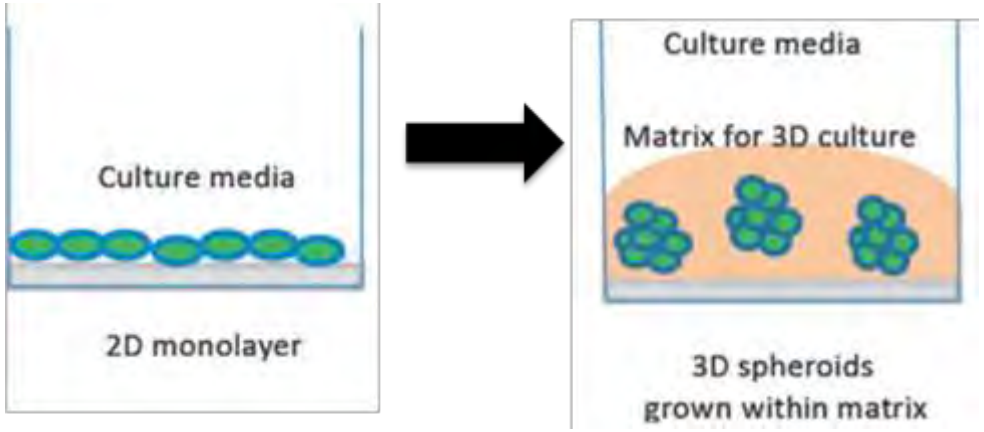
	Patient Derived Cell Lines	Patient Derived Organoids (PDOs)	Patient Derived Xenografts (PDXs)
Development Time	1 week	4-6 weeks	6-8 months
Initiation Success	Minimal	Moderate	Moderate
Cost	$	$$	$$$$
Throughput	High	Moderate	Low
Standardization	High	Low	Moderate

Bose https://doi.org/10.1016/j.medj.2021.08.005

Organoids are not the same as “tumoroids” and “spheroids”



Temporary “Spheroid” Culture

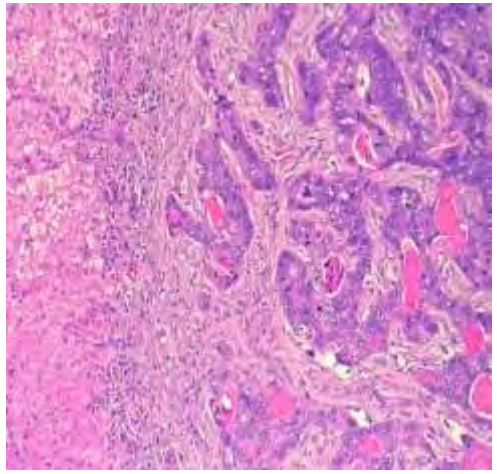


Adapted from Journal of Translational Medicine volume 19, Article number: 40 (2021)

A diverse range of terms for “organoid” cultures exist

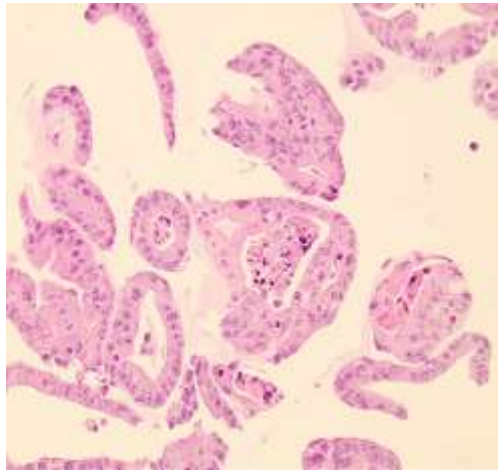
- Patient-derived organoids (PDOs) – tumor and non-tumor cultures.
- Patient-derived tumor organoids (PDTOs).
- Patient-derived organotypic tumor spheroids (PDOTS).
- Patient-derived cancer organoids (PDCOs).
- Patient-derived organotypic cancer spheroids (PDOCS).

H&E from tumor



PDO in culture

H&E from PDO

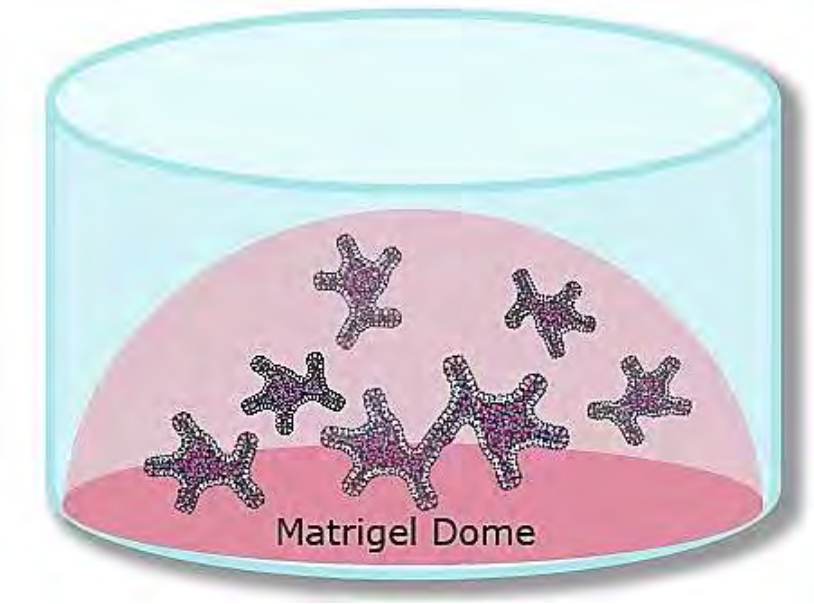


Cost is mostly derived from the labor and reagents used for culture

PDOs are embedded in a 3-dimensional matrix that is covered in specialized medium

Matrigel is the original matrix that supports the growth of organoids
- Prepared from Engelbreth-Holm-Swarm (EHS) mouse sarcomas
  - laminin (~60%), collagen IV (~30%), entactin (~8%) and the heparin sulfate proteoglycan perlecan (~2–3%).
  - complex mixture of growth factors.

Many alternative products available
- Base Membrane Extracts with or without growth factor reduction.
- Synthetic Hydrogels with added signaling proteins.



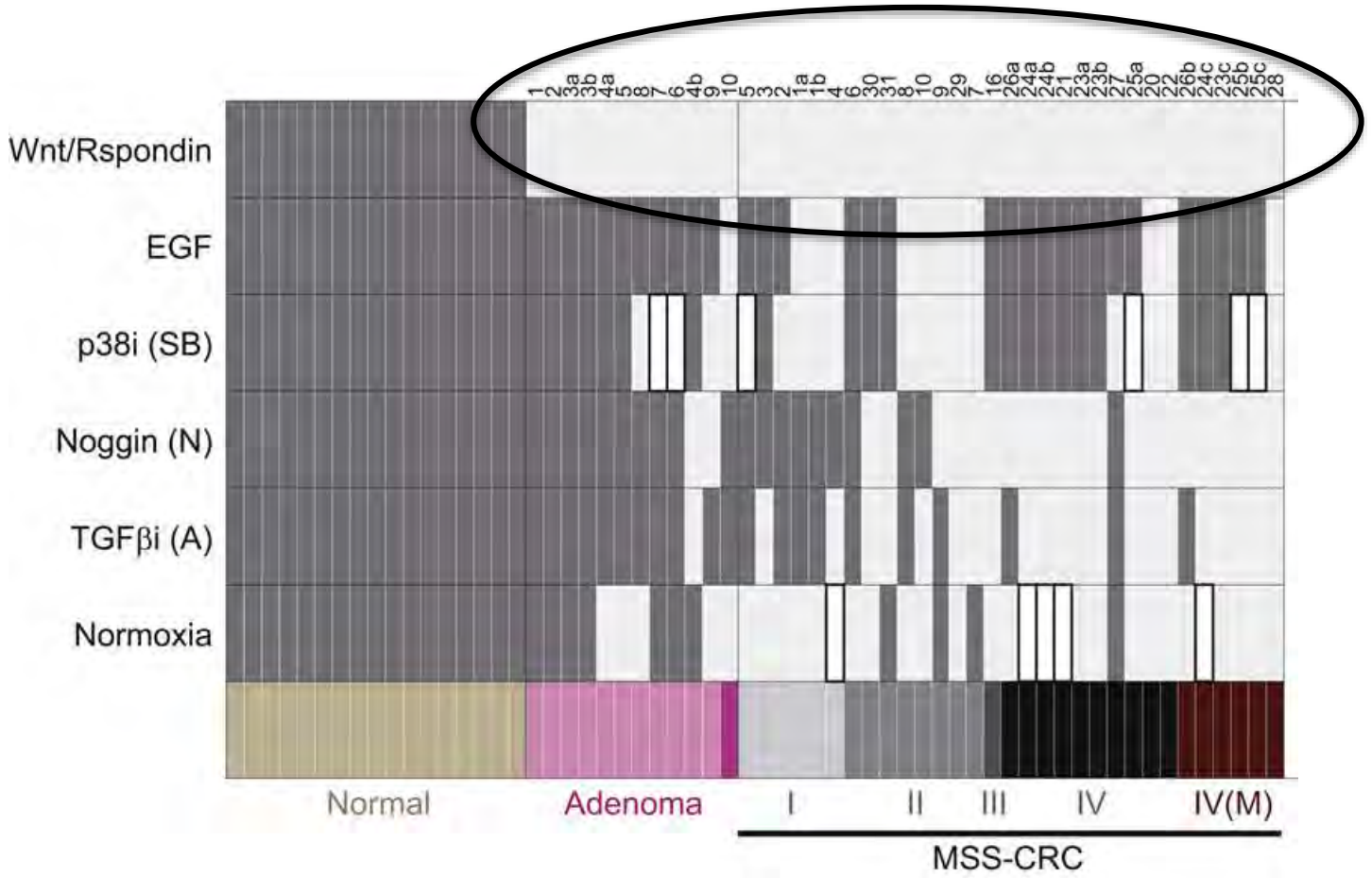
Matrices are liquid when cold (4°C) and solid at room temp and above

Specialized serum free mediums are required for each PDO cell type

- Micronutrients, amino acids, and glucose supplied by a commercially available medium.
- Requires three proteins: Wnt, Noggin, and R-spondin to maintain stemness.
- A source of lipids.
- Tissue specific growth factors are added.
- Various inhibitors to prevent apoptosis or senescence.

- Complex, but many of the components are similar and shared between different PDO media preparations.
- Specific tumor mutations can eliminate the requirement for some media components.

APC mutations make colon adenomas and adenocarcinomas independent of Wnt ligand signaling

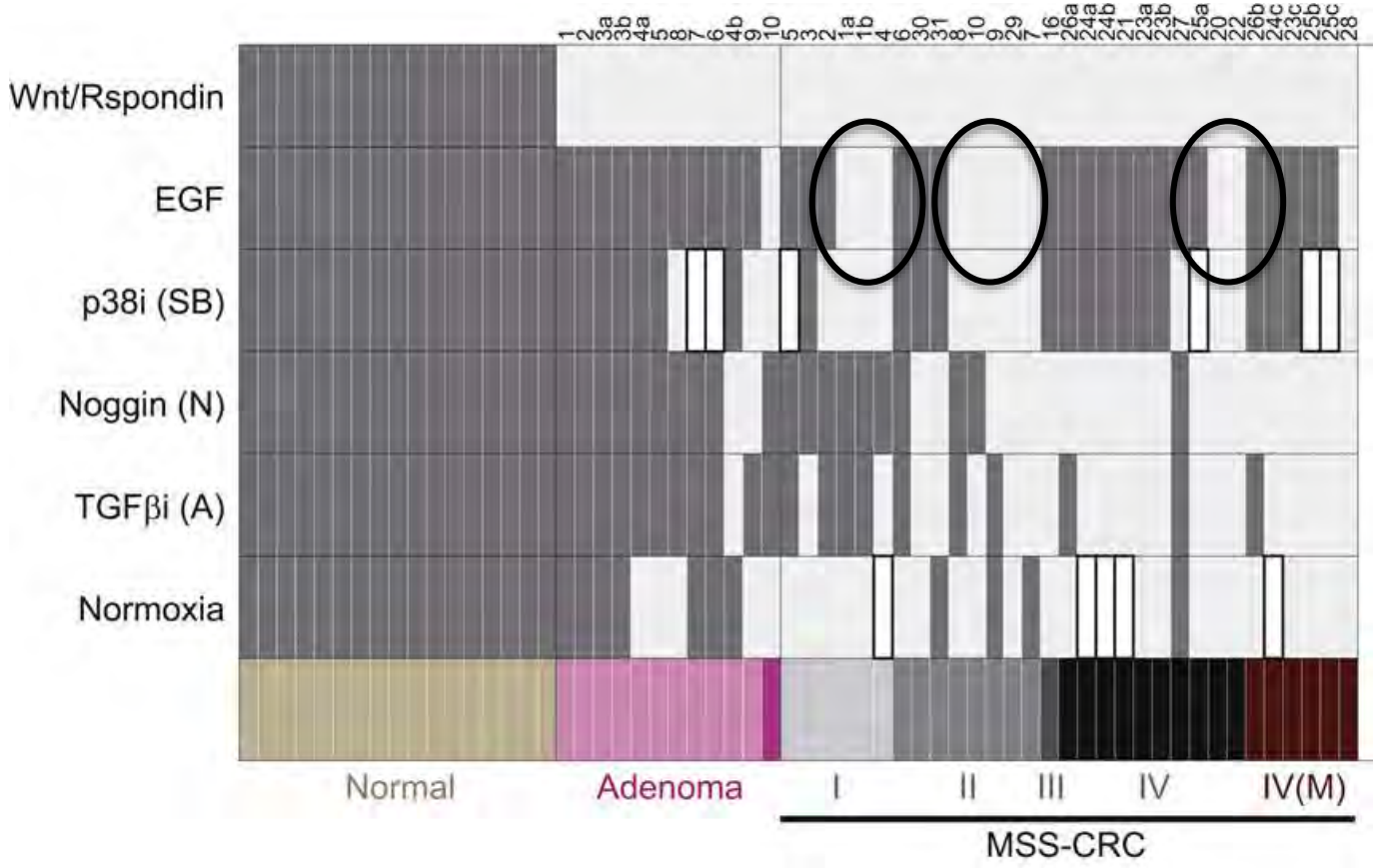


Dark Grey = required in media

Light Grey = not required

Adapted from Cell Stem Cell 2016 18827-838DOI: (10.1016/j.stem.2016.04.003)

K-Ras mutations make colon adenocarcinomas independent of EGF ligand

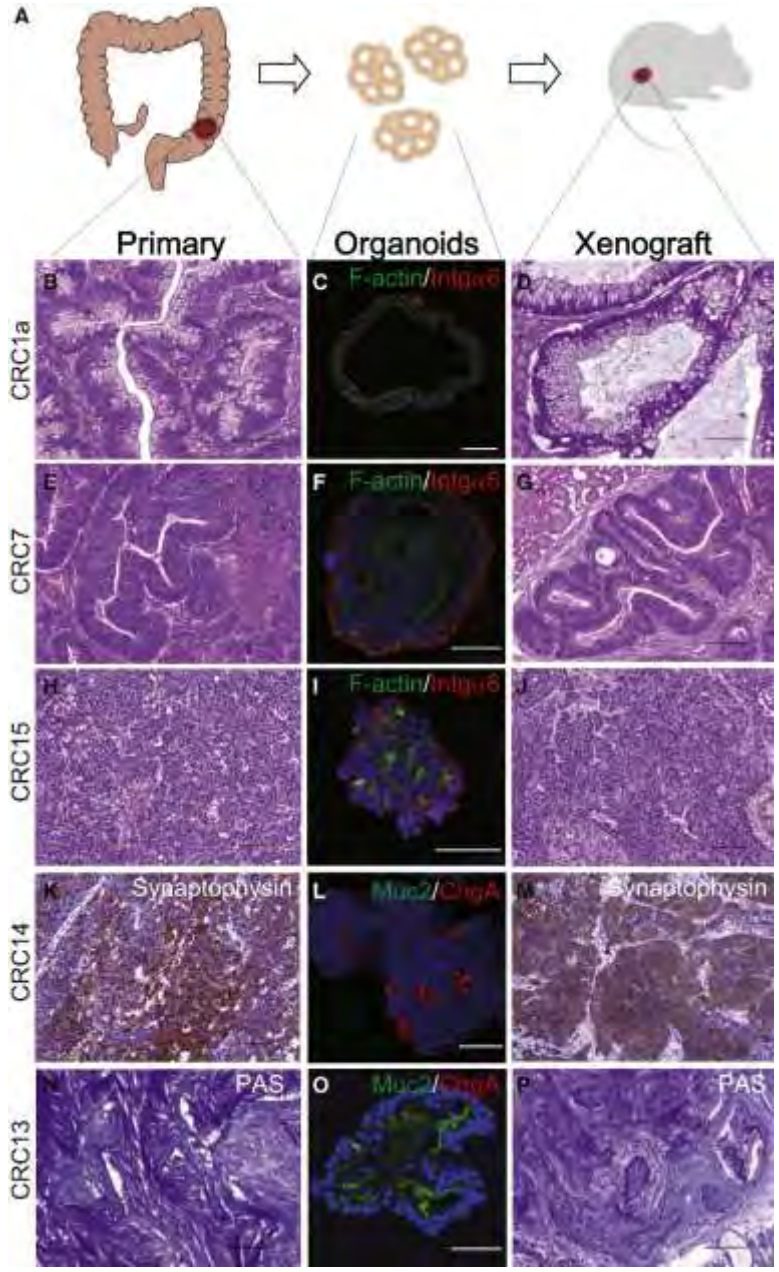


Dark Grey = required in media

Light Grey = not required

Adapted from Cell Stem Cell 2016 18827-838DOI: (10.1016/j.stem.2016.04.003)

PDOs can re-establish histologically identical tumors after re-implantation



Moderately differentiated adenocarcinoma

Moderately differentiated adenocarcinoma

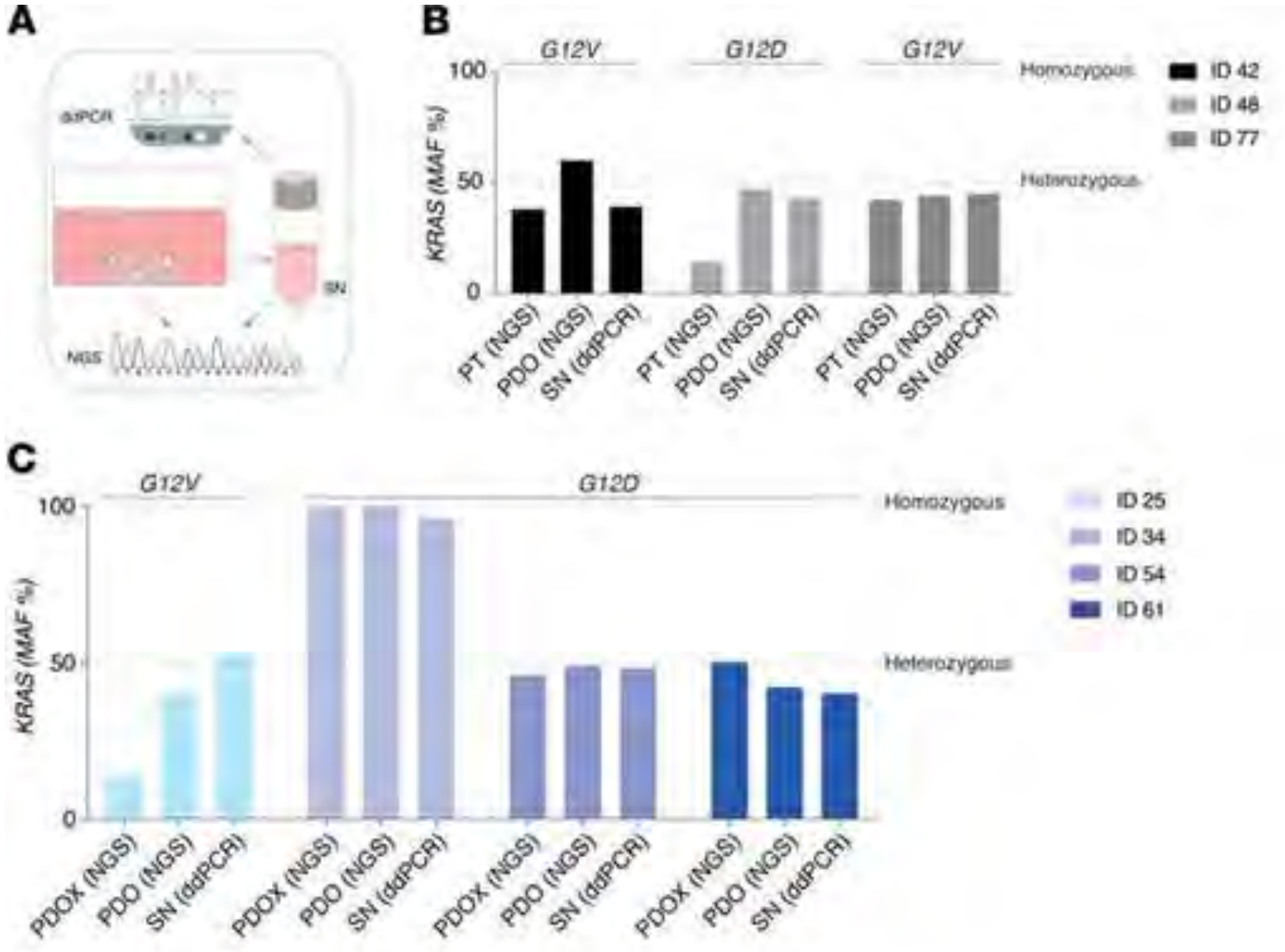
Poorly differentiated adenocarcinoma

High grade neuroendocrine carcinoma

Mucinous adenocarcinoma

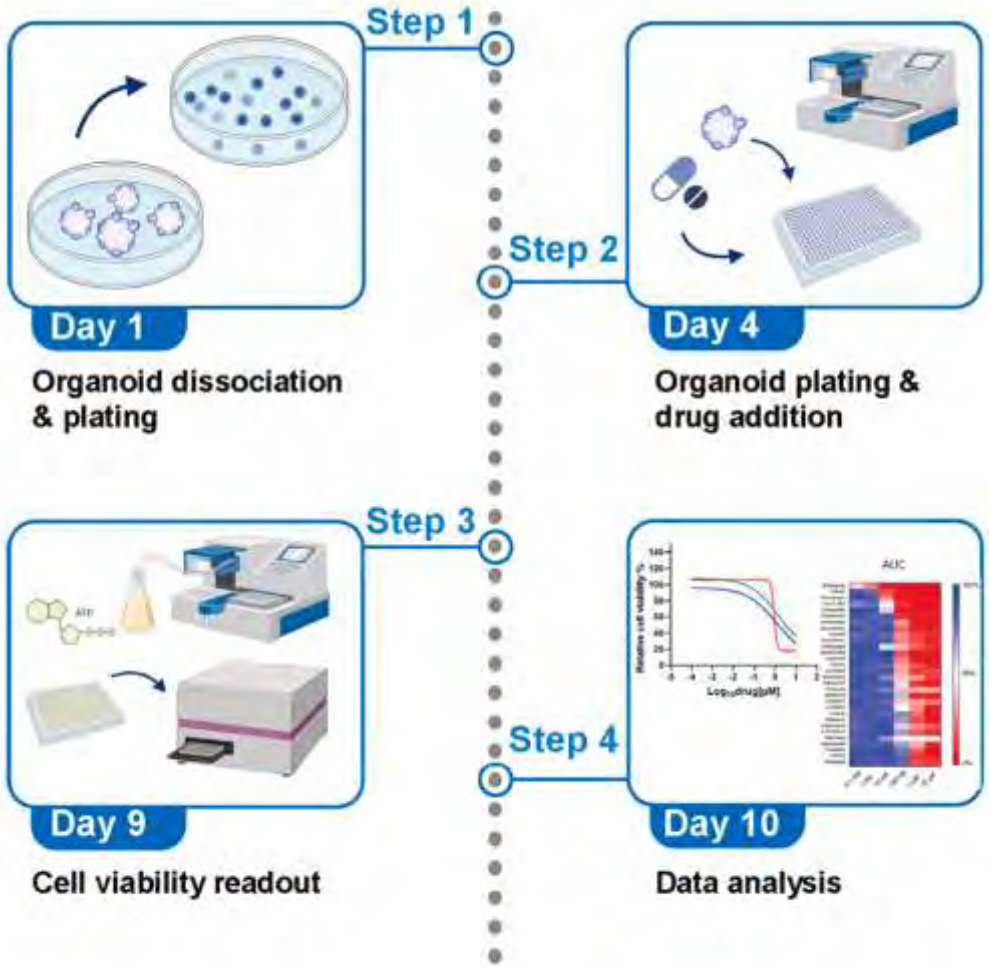
Adapted from Cell Stem Cell 2016 18827-838DOI: (10.1016/j.stem.2016.04.003)

Mutation detection from PDO closely aligns with primary tumors



JCI Insight DOI: 10.1172/jci.insight.137809

In Vitro drug screening typically measures viability after various doses of drug(s)



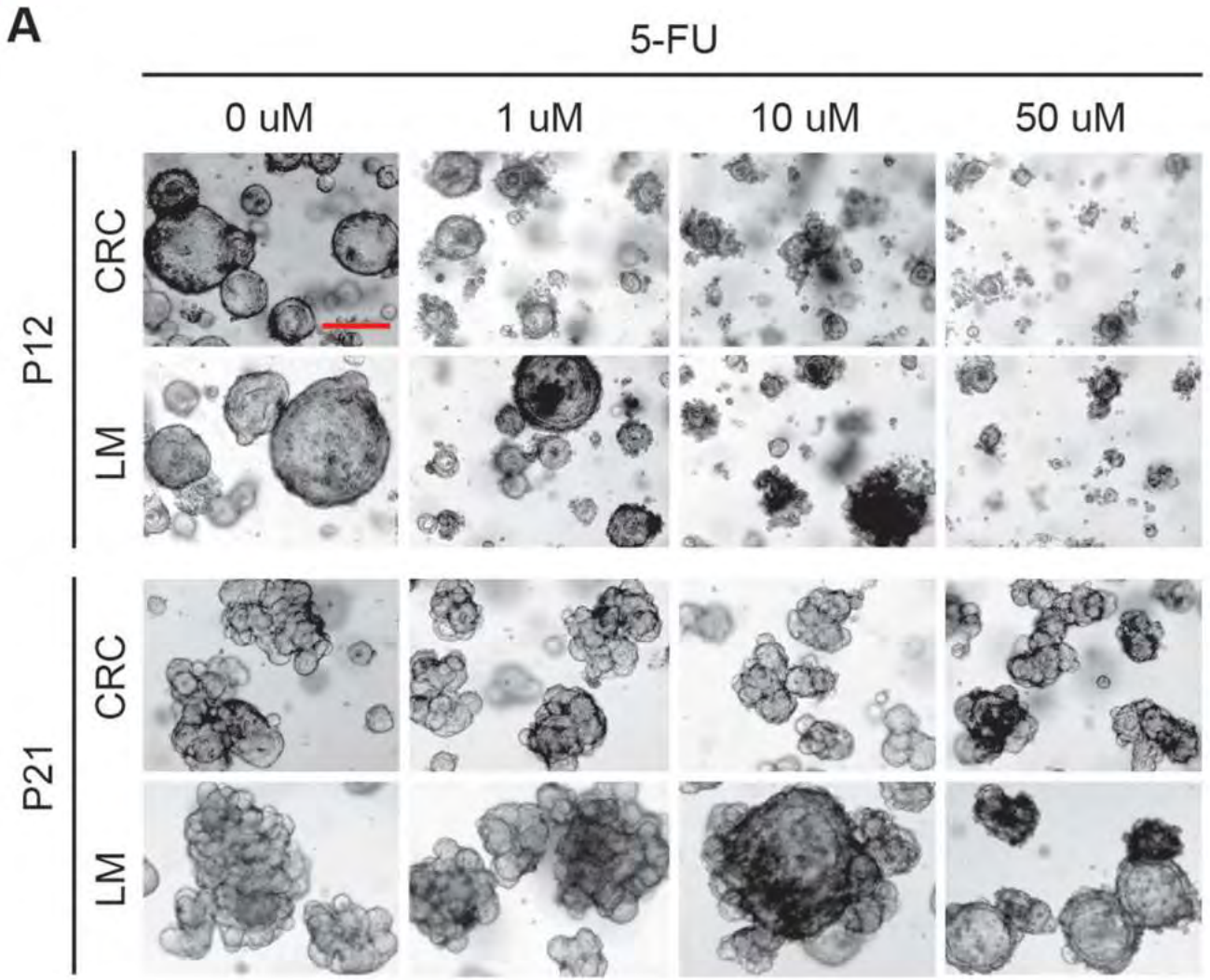
Viability determined at the end of the assay

Assess quality metrics
- Did untreated organoids grow appropriately?
- Low deviation between technical replicates?

Plot data and assess the response to each drug compared to other drug in the assay and results from prior assays.

Drost. **STAR Protocols**, 10 Jan 2022, 3(1):101079

High content measurements typically use microscopy to assess organoid response to treatment



More information,
but more complicated data
acquisition and analysis.

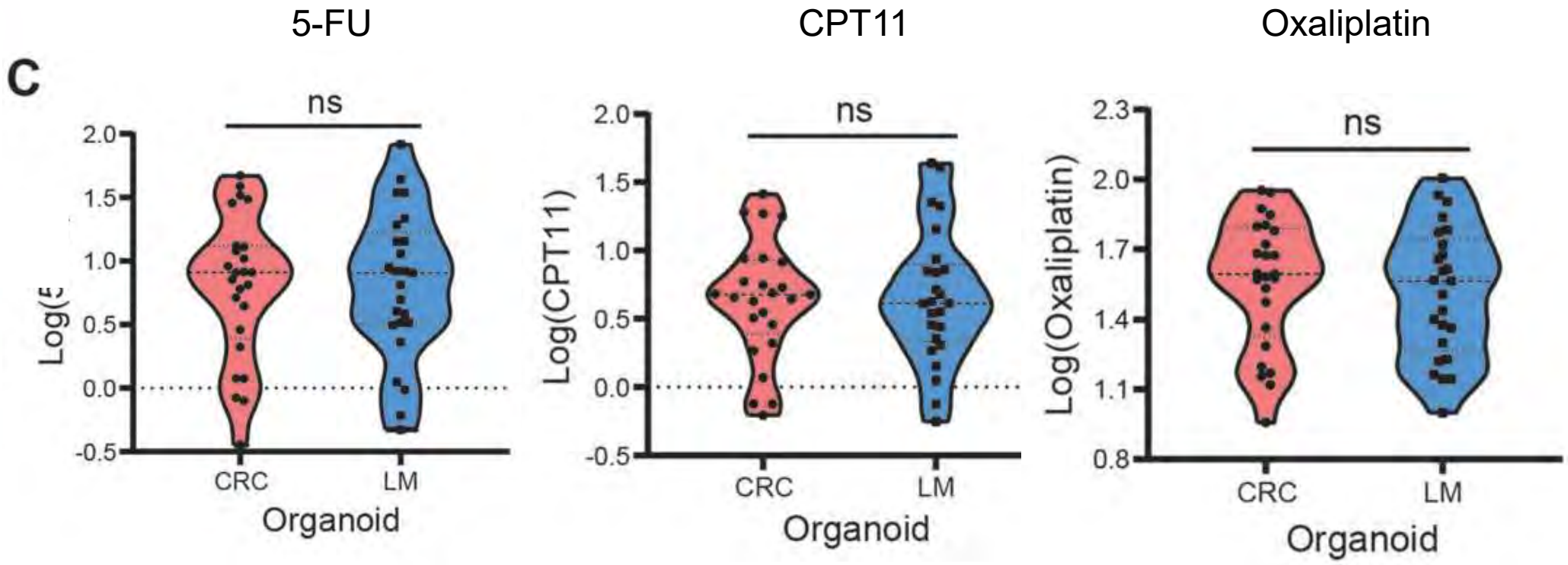
PDO responding to
drug treatment

PDO NOT responding
to drug treatment

Adv Sci (Weinh). 2022 Sep 4;e2204097. doi: 10.1002/advs.202204097.

Retrospective use of PDOs for the evaluation of patient responsiveness to standard chemotherapeutic reagents

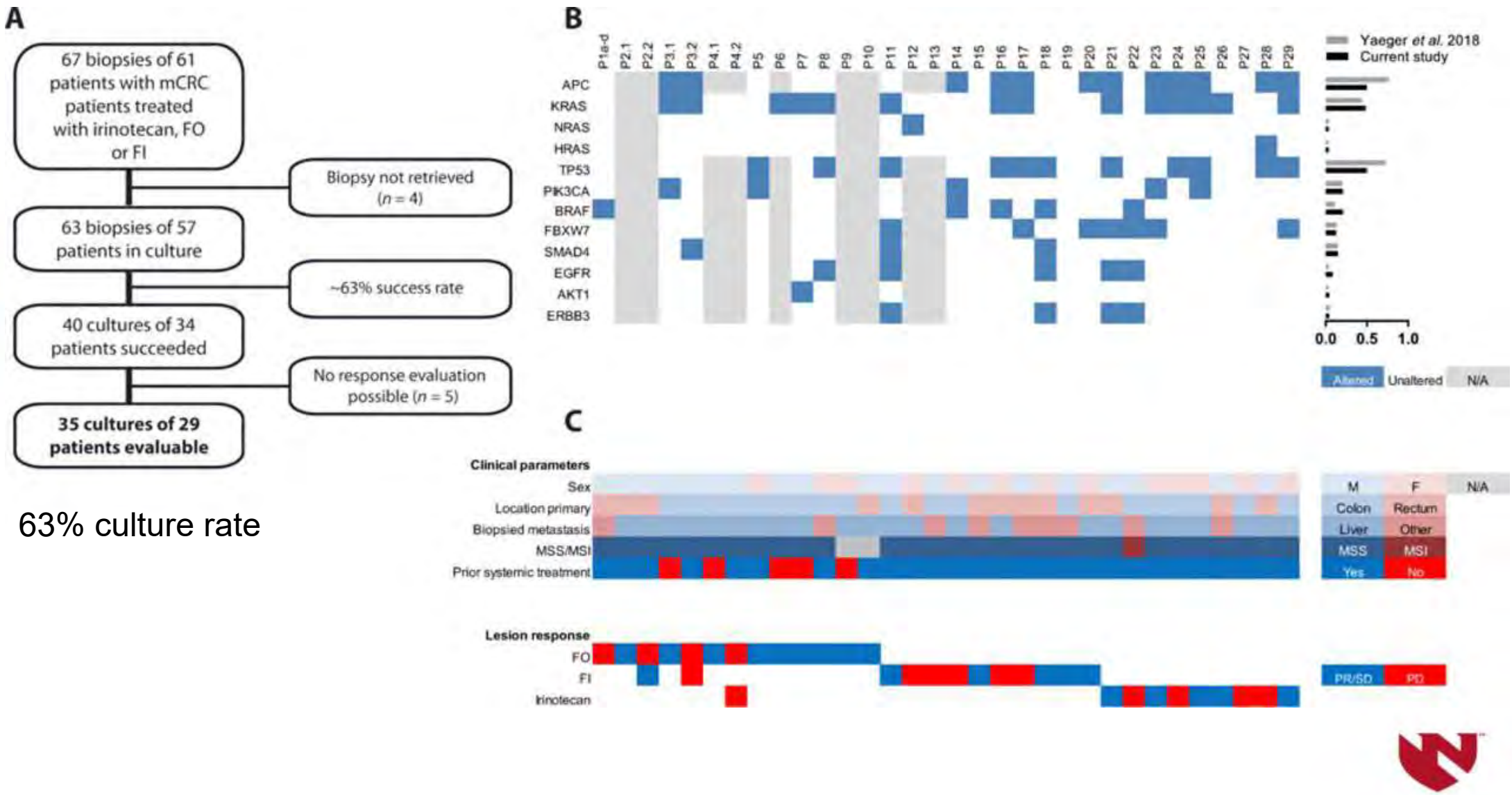
Ex Vivo PDO drug sensitivity is similar in primary CRC tumors and liver metastases



25 matching pairs of primary tumors and liver metastases showed similar sensitivities

Adv Sci (Weinh). 2022 Sep 4;e2204097. doi: 10.1002/advs.202204097.

PDOs can predict response to chemotherapy in metastatic colorectal cancer patients

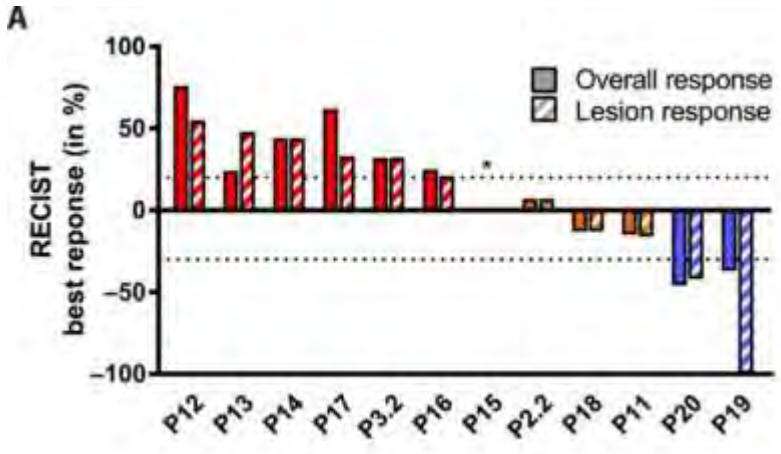


63% culture rate

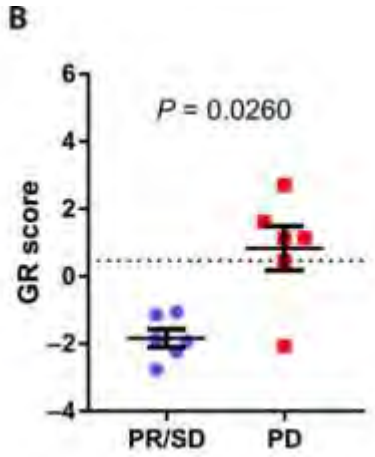
Ooft et al. SCIENCE TRANSLATIONAL MEDICINE 9 Oct 2019 Vol 11, Issue 513 DOI: 10.1126/scitranslmed.aay2574

PDOs can predict response to chemotherapy in metastatic colorectal cancer patients

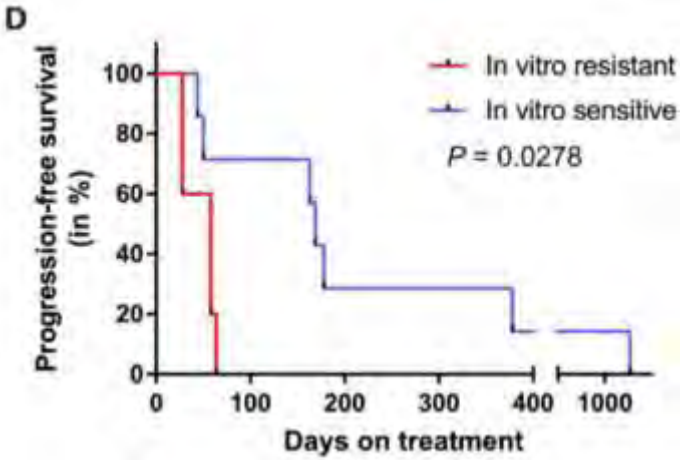
Patient tumor response by imaging



Organoid response to treatment

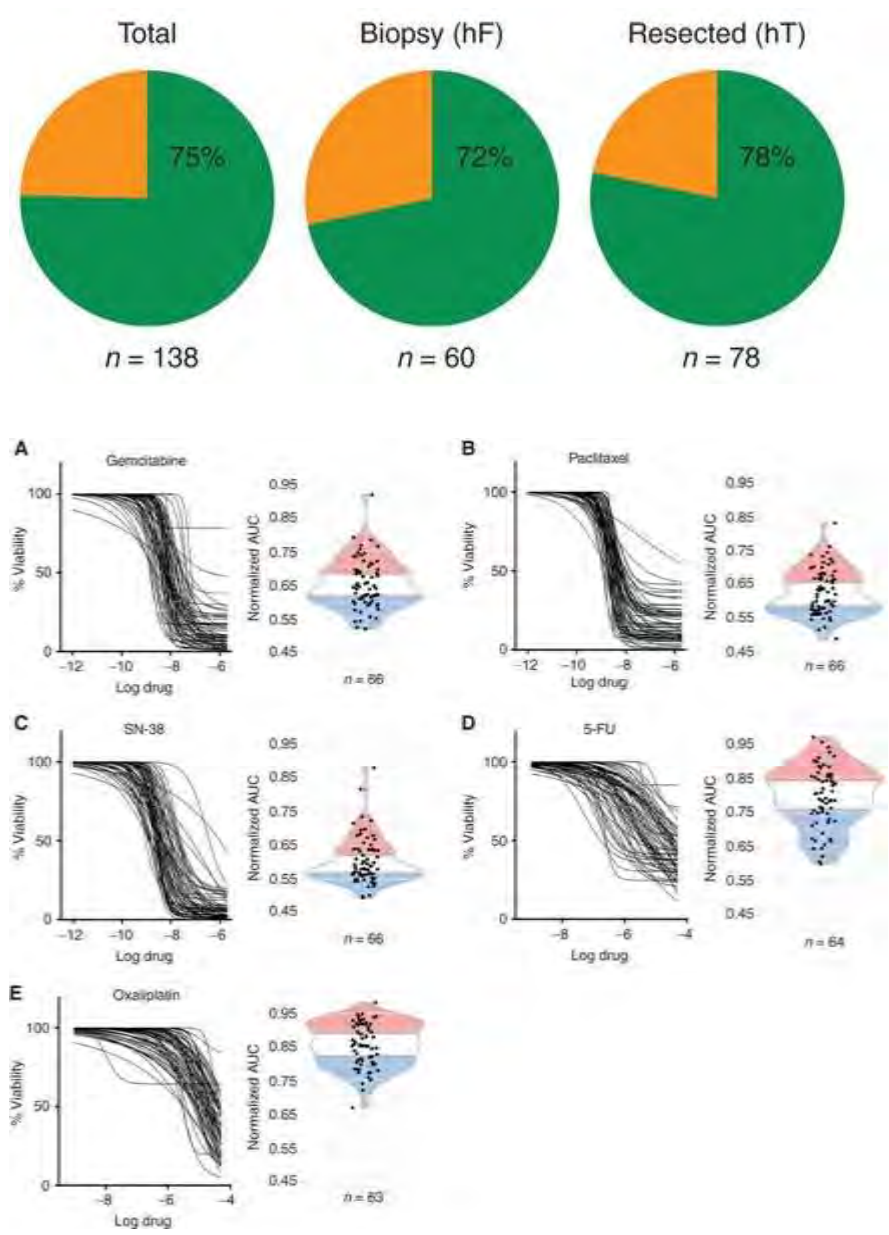


Patient time on treatment



Ooft et al. SCIENCE TRANSLATIONAL MEDICINE 9 Oct 2019 Vol 11, Issue 513 DOI: 10.1126/scitranslmed.aay2574

Organoid Profiling Identifies Common Responders to Chemotherapy in Pancreatic Cancer

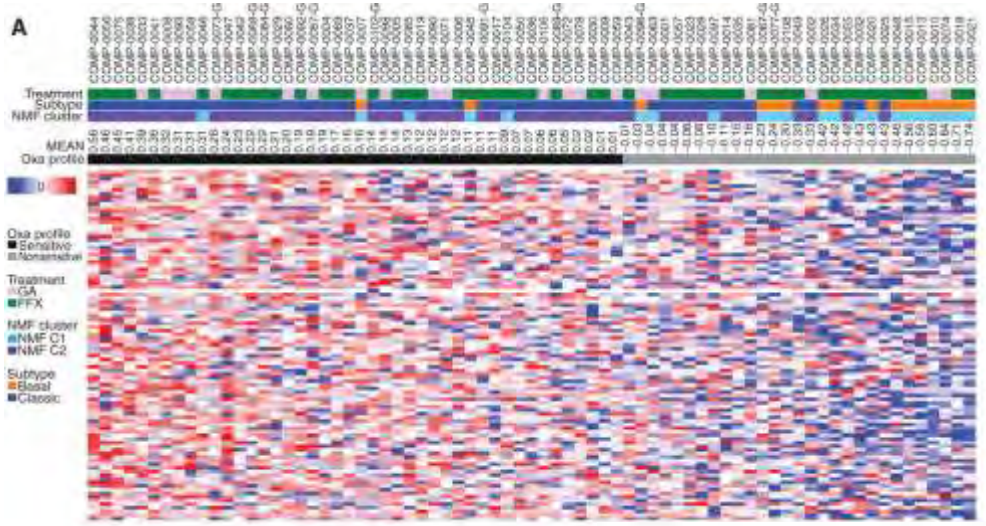


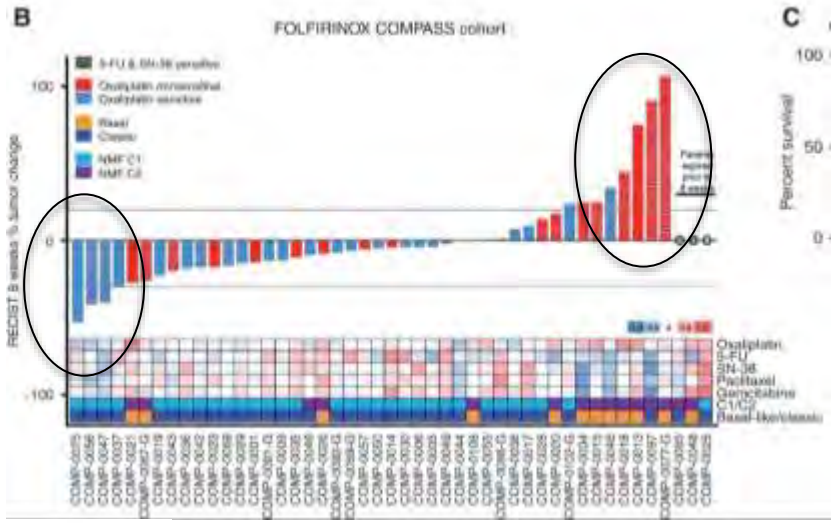
~75% culture rate

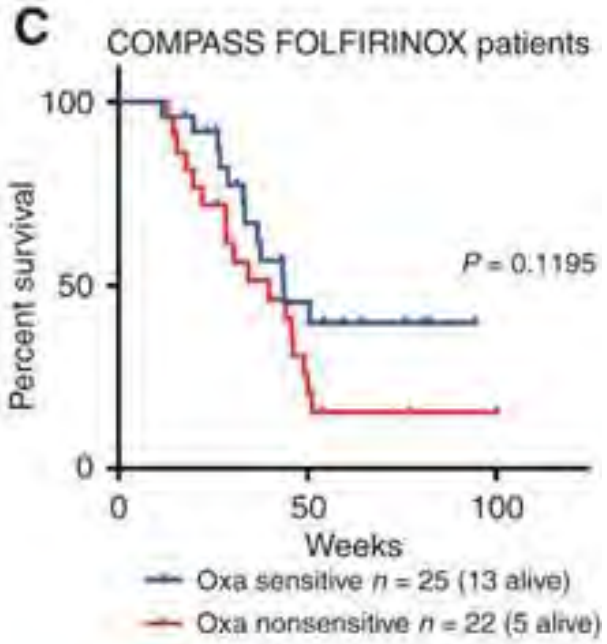
Determined the range of drug sensitives for ~65 PDOs

Cancer Discov. 2018;8(9):1112-1129. doi:10.1158/2159-8290.CD-18-0349

Development of a gene expression signature for responsiveness using PDAC PDOs



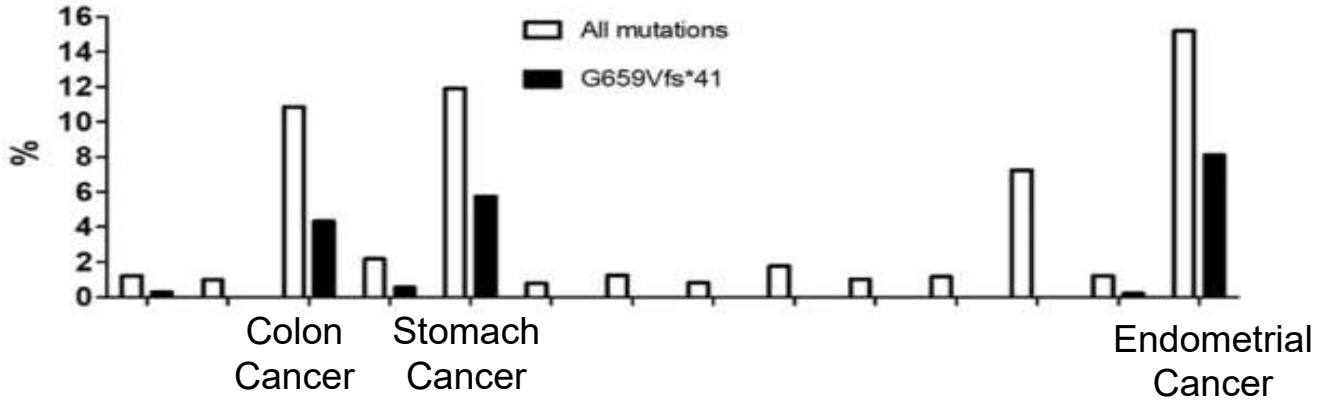


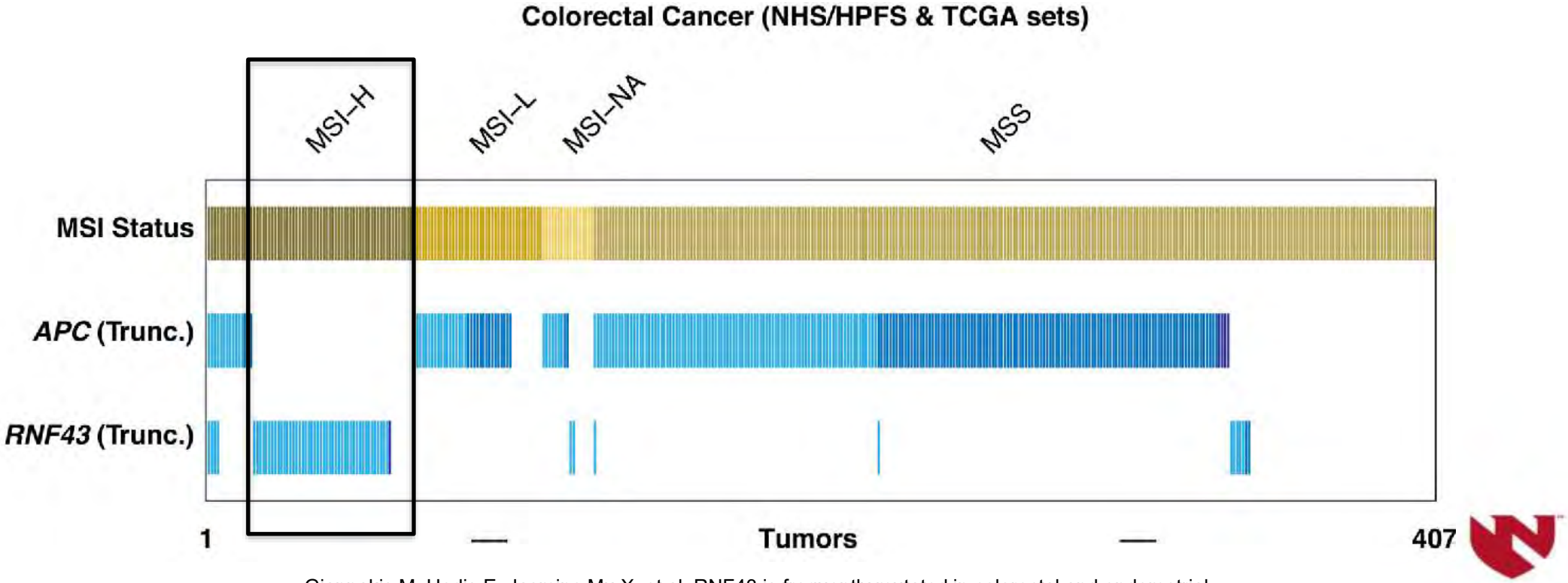


Cancer Discov. 2018;8(9):1112-1129. doi:10.1158/2159-8290.CD-18-0349

Use of PDOs for evaluation of drug sensitivities to genetic mutations

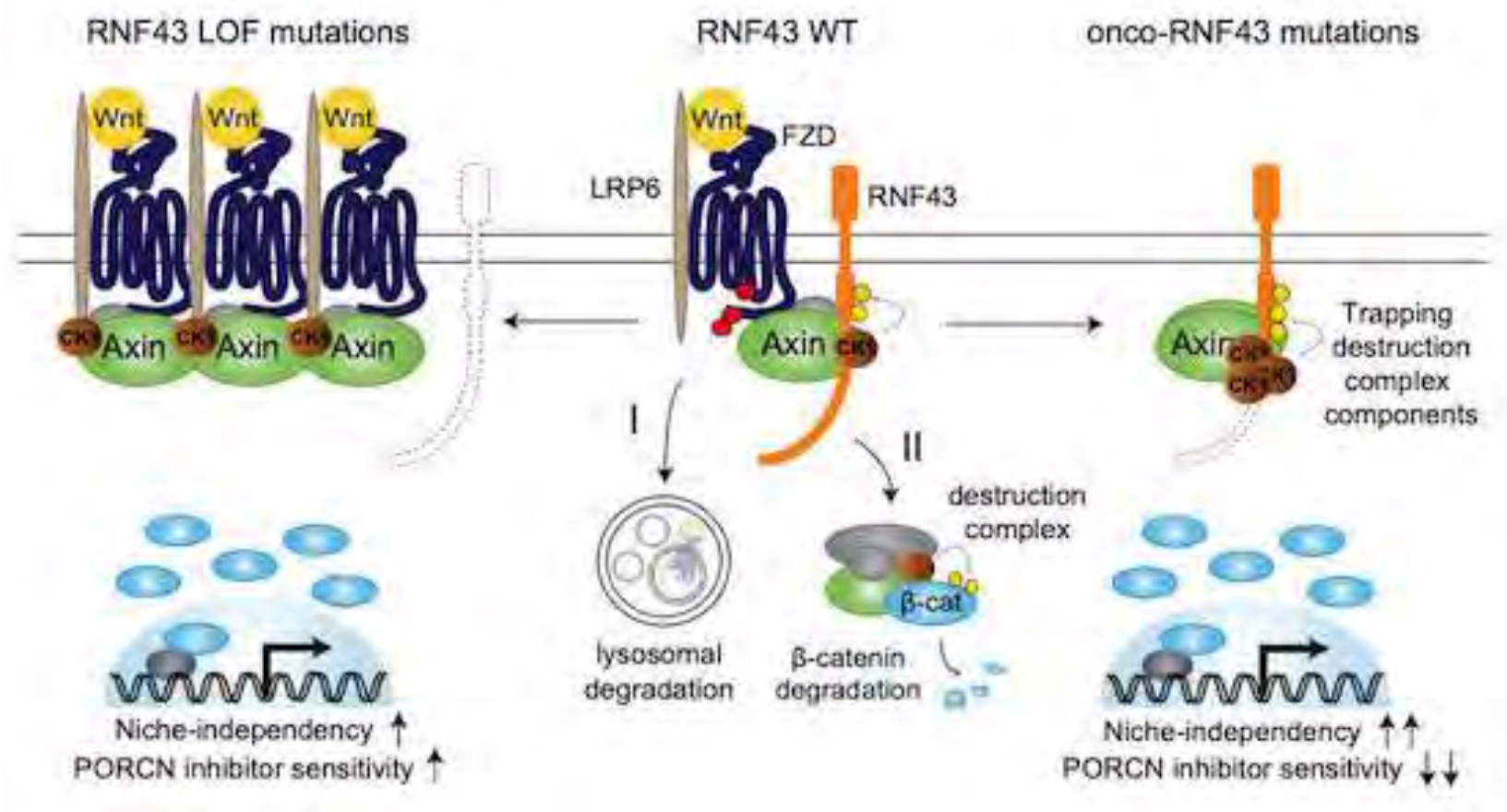
RNF43 mutations occur in the presence of Mismatch Repair deficiency and non-mutated APC





Giannakis M, Hodis E, Jasmine Mu X, et al. RNF43 is frequently mutated in colorectal and endometrial cancers. Nat Genet. 2014;46(12):1264-1266. doi:10.1038/ng.3127

RNF43 mutations promote β-catenin signaling through diverse mechanisms

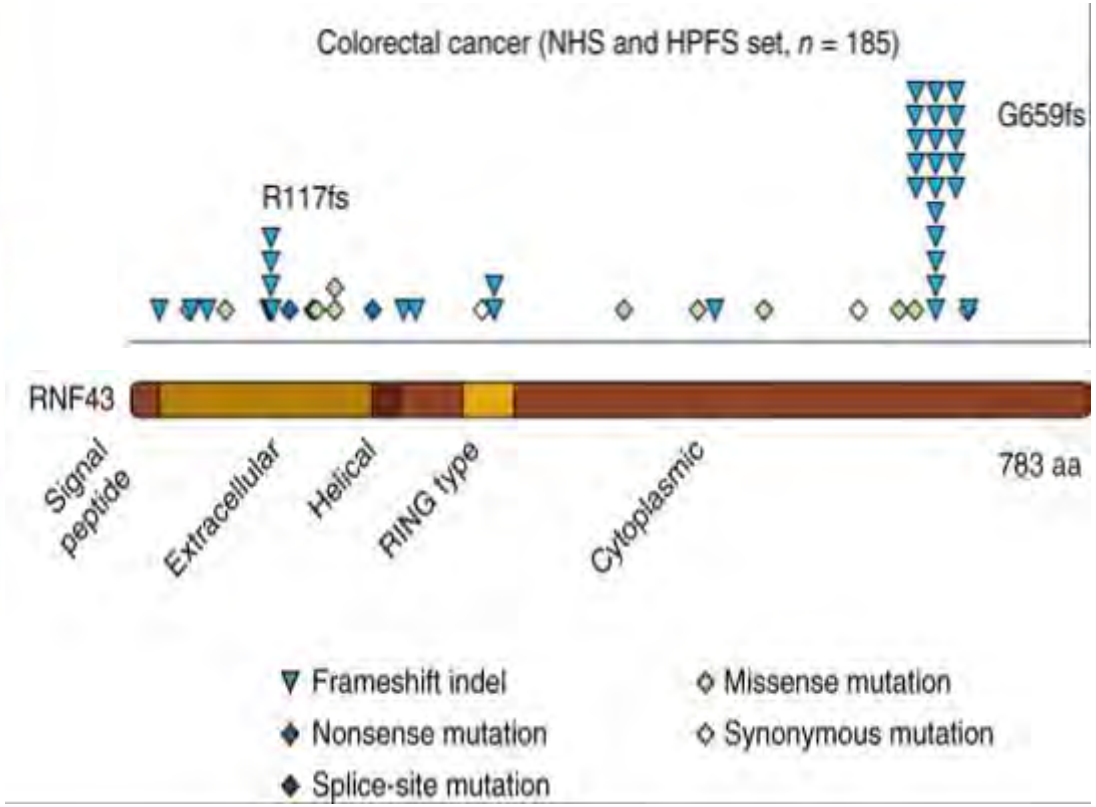


Wnt-ligand dependent

Wnt-ligand independent

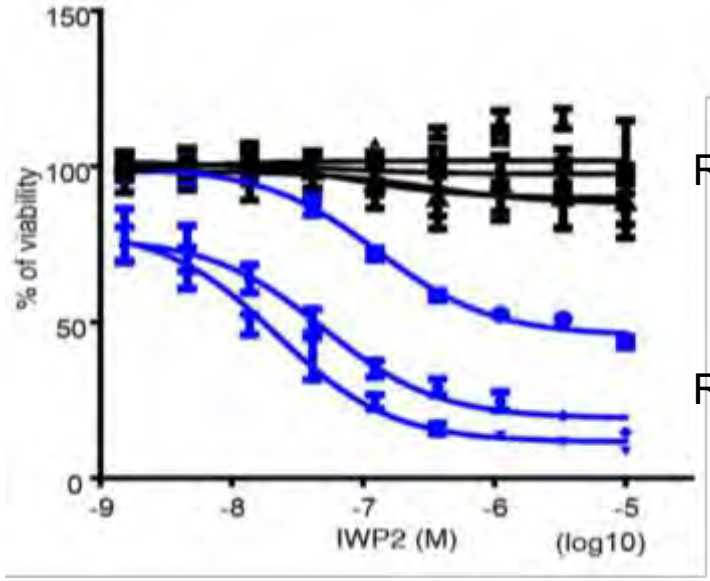
Spit M, Fenderico N, Jordens I, et al. RNF43 truncations trap CK1 to drive niche-independent self-renewal in cancer. EMBO J. 2020;39(18):e103932. doi:10.15252/embj.2019103932

RNF43 frameshift mutated organoids show vulnerability for Wnt pathway inhibitors



Giannakis M, Hodis E, Jasmine Mu X, et al. RNF43 is frequently mutated in colorectal and endometrial cancers. Nat Genet. 2014;46(12):1264-1266. doi:10.1038/ng.3127

Wnt secretion inhibitor



RNF43 Wild Type

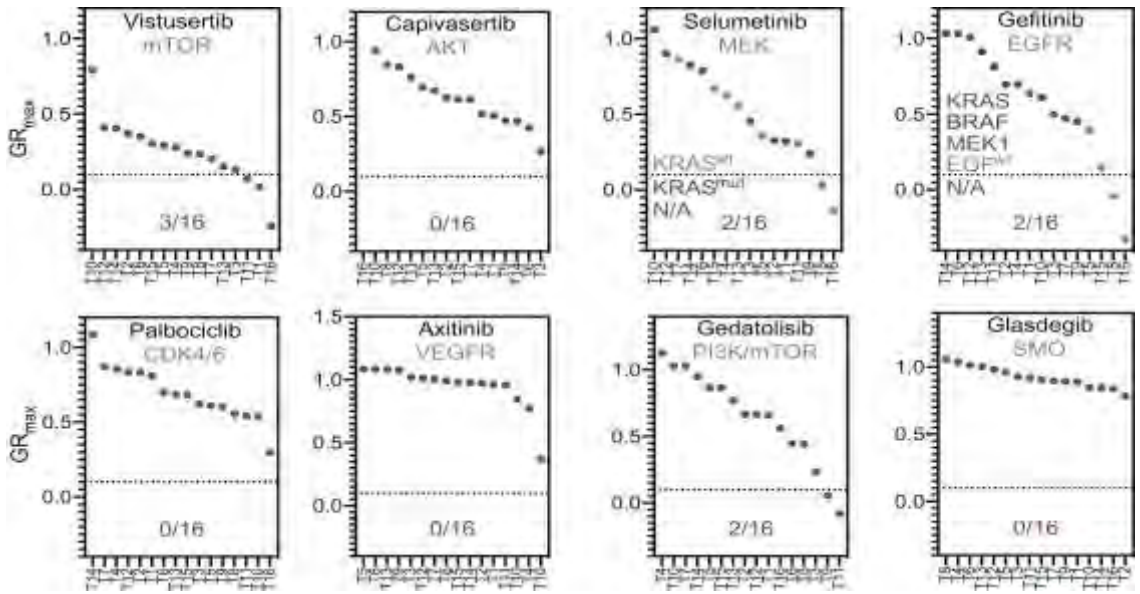
RNF43 Mutant

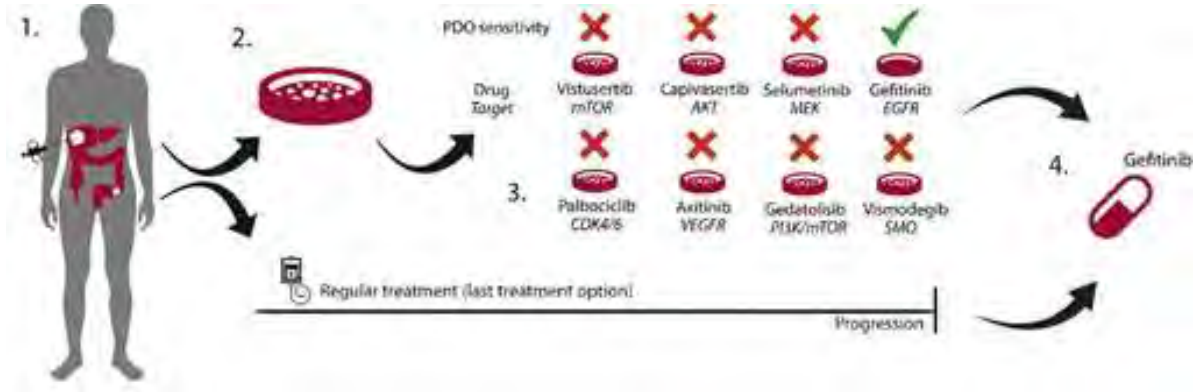
Intra-tumour diversification in colorectal cancer at the single-cell level. Nature. 2018;556(7702). doi:10.1038/s41586-018-0024-3

Prospective use of PDOs sensitivities to assign patients to experimental treatments

Prospective experimental treatment of colorectal cancer patients based on organoid drug responses (SENSOR Trial – metastatic colorectal cancer)

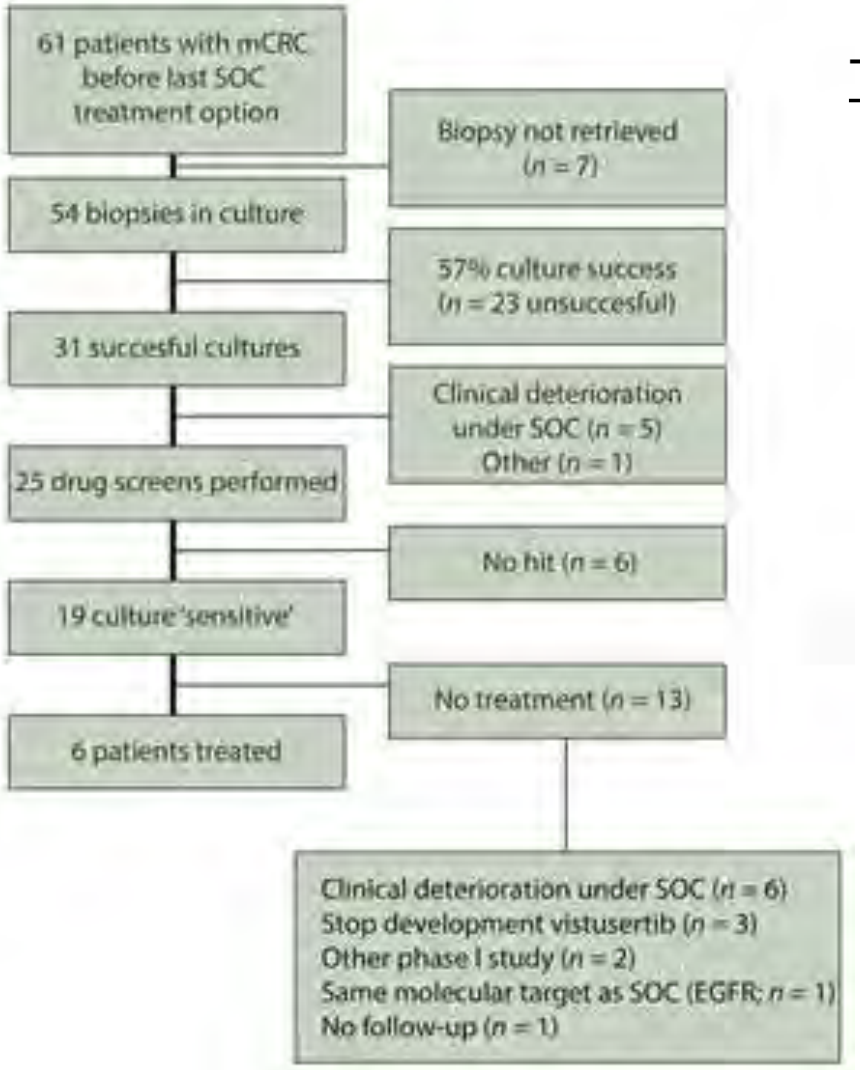
Validated screening platform informed by
prior PDO cultures treated with 8 different therapies





Ooft et al. ESMO open DOI:https://doi.org/10.1016/j.esmoop.2021.100103

SENSOR Trial – Stopped early due to limited benefit and extremely high drop out rate



<u>Trial Stopped at the Interim Analysis</u>

- 6 of 61 patients were assigned to treatment, but saw limited benefit.

- 92% drop out rate.

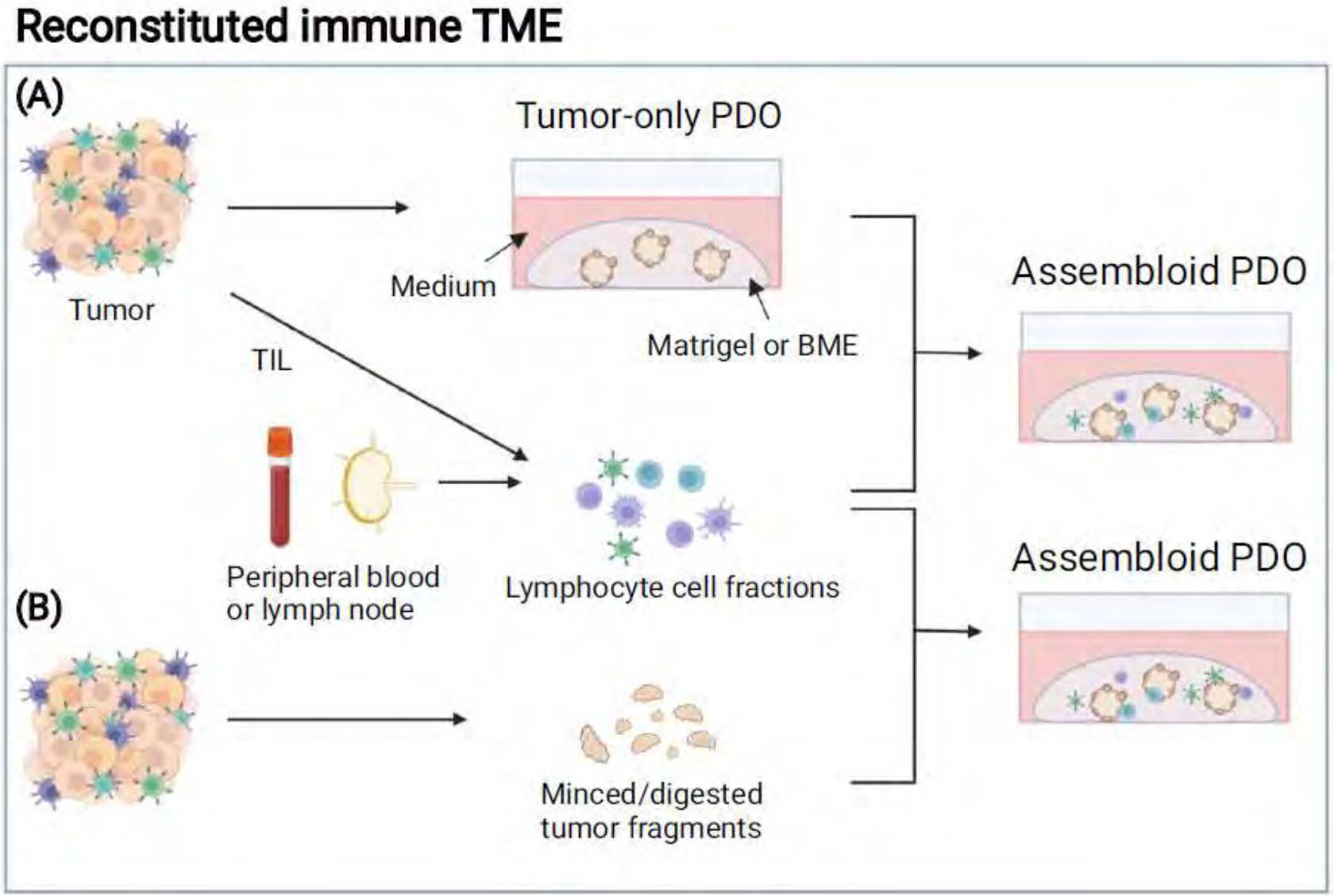
- 50% of all patients had a successful culture.

- In patients with successful PDO cultures:
  - 24% of those screen had no sensitivities.
  - 35% experienced clinical deterioration.

Ooft et al. ESMO open DOI:https://doi.org/10.1016/j.esmoop.2021.100103

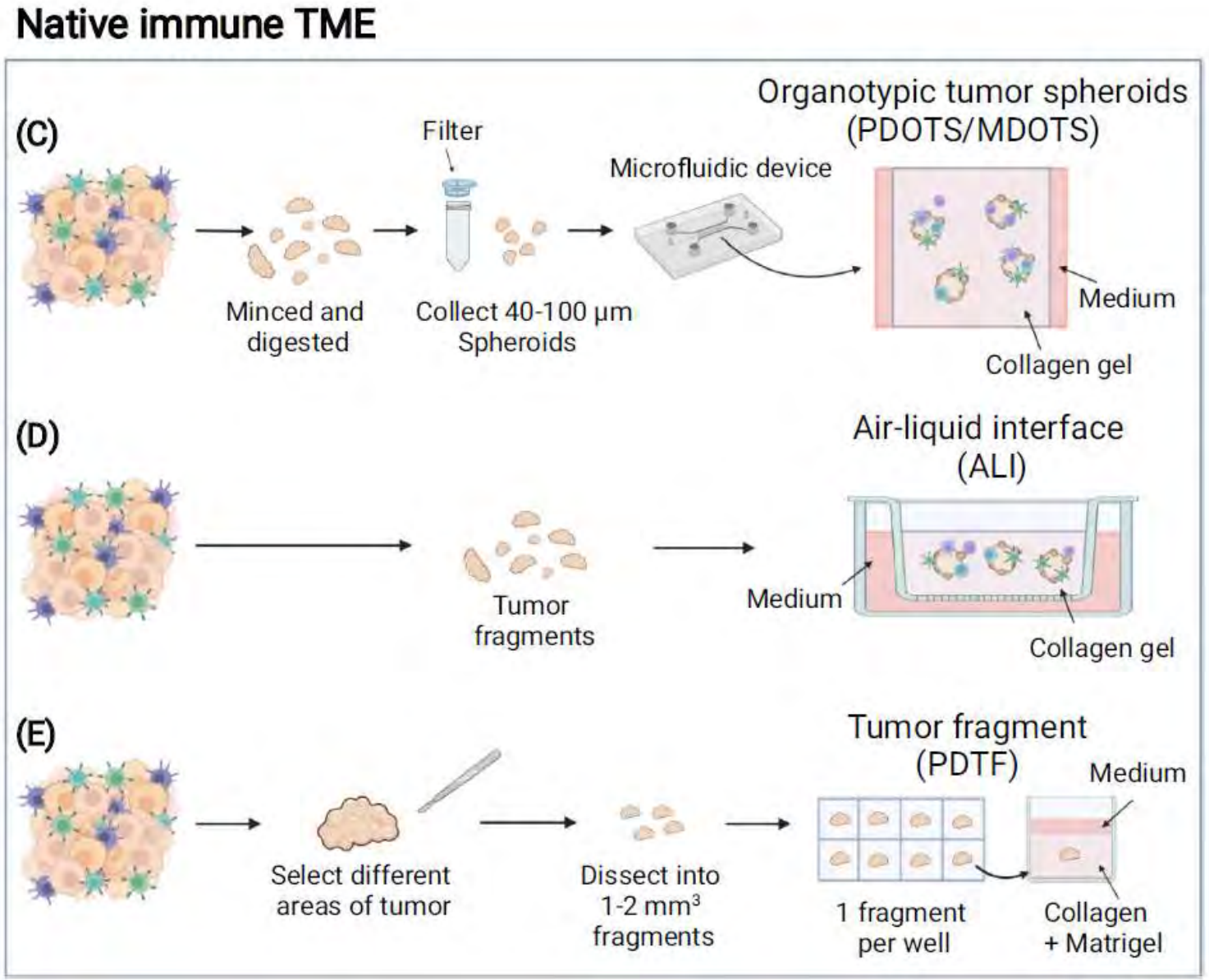
Emerging Techniques in Organoid Culture aim to model the immune system tumor microenvironment

Low throughput techniques exist for organoid culture that include the immune cells



Trends in Cancer, October 2022, Vol. 8, No. 10 https://doi.org/10.1016/j.trecan.2022.06.001

Low throughput techniques exist for organoid culture that include the microenvironment



Trends in Cancer, October 2022, Vol. 8, No. 10 https://doi.org/10.1016/j.trecan.2022.06.001