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CITATION

Bolger, Jarlath; Allen, Jonathan; Radulovich, Nikolina; Ng, Christine; Derouet, Mathieu; Shathasivam, Premalatha; et al. (2024). Patient-derived organoids for prediction of treatment response in oesophageal adenocarcinoma. Royal College of Surgeons in Ireland. Journal contribution.
<https://hdl.handle.net/10779/rcsi.25305304.v1>

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Patient-derived organoids in the treatment-naïve setting for prediction of treatment response in oesophageal adenocarcinoma

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Supplementary Methods

Organoid generation

At the index endoscopy, biopsies were taken and stored in PBS containing Penicillin-Streptomycin-Neomycin, Antibiotic-Antimycotic (Gibco, USA) and Primocin (Invivogen, USA) (PBS PNP). The biopsies were then cut into small pieces using a scalpel and washed 3 times in PBS PNP. The remaining pellet was resuspended in freshly made digestion medium containing Collagenase type II (5 mg/ml) (Gibco, USA), DNase I (100 ug/ml) (Sigma, USA), and Y-27632 dihydrochloride (10 uM) (Tocris, USA) in advanced DMEM/F12 and transferred to a 15 mL tube. The tube was then incubated for 1 h at 37°C on a shaking platform (120 rpm). The tissue was then washed and remaining pellet incubated with TrypLE Select Enzyme (10X) (Gibco, USA) and Y-27632 dihydrochloride (10 uM) for 10 min at 37°C on a shaking platform (120 rpm). Digestion was stopped by adding fresh advanced DMEM/F12 containing 20% fetal bovine serum (Thermo Scientific, USA). The digested sample was filtered through a 70 µm cell strainer and cells spun down. The pellet was resuspended in Matrigel GFR (Corning, USA) to a concentration of 30,000 cells per 50 µL of Matrigel. 50 µL of cell suspension in Matrigel was plated per well of a 24-well plate (Thermo Scientific Nunc, USA) and incubated for 10 min at 37 °C for polymerisation after which either Medium A or B was added to the wells. Medium was changed every three to four days with freshly made medium. After passage 4, surviving organoids were STR tested. All organoids are authenticated with short tandem repeat profiling. Representative samples of shallow whole exome sequencing demonstrating significant copy number variations are in supplemental Figure 1. Whole exome sequencing was performed as previously described¹.

Medium A: Advanced DMEM/F12, HEPES (10 mM), GlutaMax (2 mM) (Gibco, USA), Penicillin-Streptomycin-Neomycin (1x), Primocin (50 µg/ml), Antibiotic-Antimycotic (1x), N-Acetyl L-Cysteine (1.25 mM) (Sigma, USA), B-27 Supplement (1x) (Gibco, USA), Gastrin (10 nM) (Sigma, USA), EGF (50 ng/mL), Noggin (100 ng/mL) (R&D Systems, USA), A-83-01 (0.5 µM) (Tocris, USA), CHIR 99021 (2.5 µM) (R&D Systems, USA), RSpondin1 conditioned medium (10% v/v), Wnt-3A conditioned medium (40% v/v). The conditioned media were obtained from Princess Margaret Living Biobank. Medium B: Medium A + SB202190 (10 µM) (Sigma, USA).

Organoid drug testing

For *in vitro* drug testing, chemotherapy agents were purchased from Selleckchem (Houston, TX) and dissolved according to manufacturer's recommendation. Organoids were dissociated to single cells, counted, and seeded onto a thin layer of Matrigel in 384 well plates (3,000 cells per well) in triplicate for 3 days prior to drug treatment. Organoids were treated with 12 points of drug concentrations (0.01–10 µM) for 96 h and cell viability was assessed using the CellTiter-Glo 3D assay (Promega, USA). Relative IC₅₀ values were graphed and calculated from 12-point drug concentrations with four-parameter nonlinear logistic equation using using 'drc' for R and *jamovi* (version 1.6, retrieved from <https://www.jamovi.org>), with 'deathwatch' and 'jsurvival' modules. IC₅₀ was calculated using drc by generating a dose response model with a continuous type response.

Supplementary Figures and Tables

Patient number	Organoid passage number	Age	Tumour location	cTNM	Neoadjuvant therapy	Dose modifications/cycles completed	Surgical approach	ypTNM	TRG
1	6	76	Siewert II	cT3N0M0	CROSS	41.4Gy administered, completed chemotherapy as prescribed.	Ivor Lewis MIO	ypT2N1M0	1
2	24	67	Siewert II	cT3N0M0	CROSS	41.4Gy administered, completed chemotherapy as prescribed.	Open extended total gastrectomy	ypT1bN2M0	1
3	7/9*	59	Siewert II	cT3N1M0	CROSS	41.4Gy administered, completed chemotherapy as prescribed.	Ivor Lewis MIO	ypT3N2M0	2
4	9	54	Siewert I	cT2N1M0	CROSS	41.4Gy administered, completed chemotherapy as prescribed.	Ivor Lewis MIO	ypT2N2M0	2
5	14	30	Siewert III	cT4N1M0	FLOT	4 cycles, docetaxel held for last 2 cycles due to liver toxicity	Extended total gastrectomy	ypT3N3M0	3
6	13	62	Siewert I	cT3N0M0	CROSS	41.4Gy and 4 cycles of chemotherapy, 1 held due to neutropenia	Ivor Lewis MIO	ypT3N1M0	3
7	11	58	Siewert I	cT3N3M0	CROSS	41.4Gy administered, completed chemotherapy as prescribed.	Ivor Lewis MIO	ypT2N2	1
8	9	65	Siewert I	cT3N1M0	CROSS	41.4Gy and 4 cycles of chemotherapy, last held due to neutropenia.	Ivor Lewis MIO	ypT2N0M0	1
9	5	71	Siewert II	cT3N1M0	CROSS	41.4Gy administered, completed chemotherapy as prescribed.	Ivor Lewis MIO	ypT3N1M0	3
10	5	62	Siewert II	cT3N3M0	FOLFOX	Received 6 cycles, dose reduction of 50% for oxaliplatin and 5-FU due to thrombocytopenia.	Ivor Lewis MIO	ypT3N0M0	2
11	8	58	Siewert III	cT3N1M0	FLOT	Received 4 cycles, 20% dose reduction for cycles 3 and 4 due to toxicities.	Open total gastrectomy	ypT4aN1M0	2
12	5	79	Siewert I	cT3N0M0	CROSS	41.4Gy administered, completed chemotherapy as prescribed.	Ivor Lewis MIO	ypT2N1M0	2
13	6	72	Siewert I	cT3M3N0	CROSS	41.4Gy administered, completed	Ivor Lewis MIO	ypT3N1M0	2

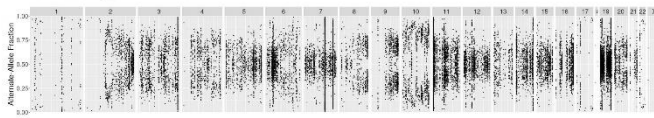
						chemotherapy as prescribed.			
14	5	66	Siewert II	cT3N1M0	CROSS	41.4Gy administered, final 2 cycles of chemotherapy omitted due to febrile neutropenia	Ivor Lewis MIO	ypT3N3M0	3
15	12	60	Siewert III	cT3N2M0	FLOT	Received 4 cycles without dose reduction. Cycle 3 delayed by 1 week due to elevated liver enzymes.	Open extended total gastrectomy	ypT3N2M0	2
16	15	54	Siewert II	cT3N1M0	FLOT plus chemoradiation	Received 3 cycles of FLOT plus 45Gy with concurrent capecitabine.	Ivor Lewis MIO	ypT3N0M0	1
17	12	67	Siewert III	cT3N0M0	CROSS	41.4Gy administered, completed chemotherapy as prescribed.	Open total gastrectomy	ypT2N0M0	1
18	6	53	True gastric cancer	cT3N2M0	FLOT	Received 4 cycles as prescribed	Laparoscopic gastrectomy	ypT4aN3M0	3
19	8	45	Siewert II	cT3N1M0	CROSS	41.4Gy administered, completed chemotherapy as prescribed.	McKeown MIO	ypT3N2M0	2
20	6	63	Siewert II	cT3N0Mo	CROSS	41.4Gy administered, completed chemotherapy as prescribed.	Ivor Lewis MIO	ypT2N0M0	2
21	7	62	Siewert I	cT3N2M0	CROSS	41.4Gy administered, completed chemotherapy as prescribed.	Ivor Lewis MIO	ypT2M0N0	1
22	7	67	Siewert II	cT3N0M0	CROSS	41.4Gy administered, completed chemotherapy as prescribed.	Ivor Lewis MIO	ypT1aN0M0	1
23	6	64	Siewert I	cT3N1M0	CROSS	41.4Gy administered, completed 4 cycles chemotherapy, one omitted due to cardiac toxicity.	Ivor Lewis MIO	ypT3N2M0	3

Supplementary Table 1: Patient characteristics of oesophageal cancer patients treated on a curative pathway. *cTNM*=clinical tumour, node and metastasis staging; *ypTNM*=final pathological TNM classification after neoadjuvant therapy. *TRG*=tumour regression grade as described by the College of American Pathologists. *Organoids at passage 7 were treated with cisplatin and paclitaxel; organoids at passage 9 were also treated with paclitaxel.

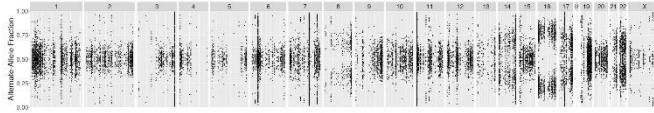
Patient	Organoid passage number	Age	Tumour Location	Metastatic site(s)	First line chemotherapy	Response at 3 and 6 months	Second line chemotherapy	Response at 3 and 6 months
1	10	75	GOJ	Liver and supra-clavicular nodes.	Cisplatin, capecitabine, trastuzumab	Stable disease	N/A, palliation	-
2	9	65	GOJ	Diffuse and distant lymphadenopathy.	Cisplatin, gemcitabine, trastuzumab	Progressive disease	ZW25 trial	Progressive disease
3	5	41	GOJ	Lung, liver.	FOLFOX	Partial response	FOLFIRI	Progressive disease
4	5	54	Gastric cancer	Peritoneum, diffuse lymphadenopathy and locally invasive to diaphragm.	FOLFOX	Partial response	FOLFIRI	Stable disease
5	7	52	GOJ	Liver and distant lymphadenopathy.	Cisplatin, 5-FU, trastuzumab	Partial response	ZW25 trial	Partial response
6	8	57	Gastric cancer	Peritoneum, distant lymphadenopathy.	FOLFOX and nivolumab	Partial response	ZW25 trial	Progressive disease
7	20	67	GOJ	Lung and peritoneum.	FOLFOX and nivolumab	Stable disease	FOLFIRI	Stable disease
8	9	62	GOJ	Liver, diffuse lymphadenopathy, peritoneum.	CAPOX +/- pembrolizumab	Partial response	FOLFIRI	Progressive disease

Supplementary Table 2: Characteristics of patients presenting with metastatic disease treated with palliative intent. *GOJ*= gastro-oesophageal junction. Disease responses are with RECIST criteria, taken 3 and 6 months after commencing first- or second-line chemotherapy.

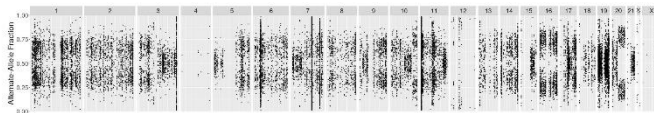
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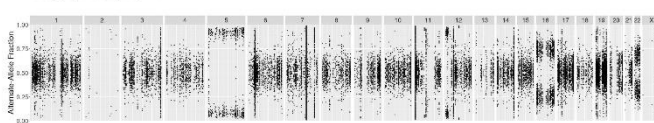
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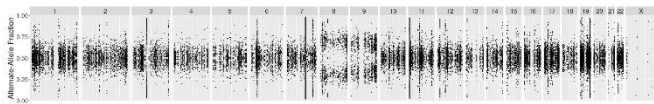
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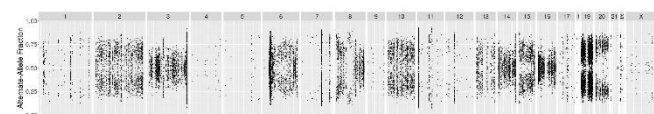
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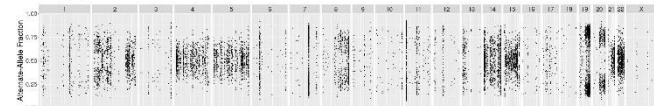
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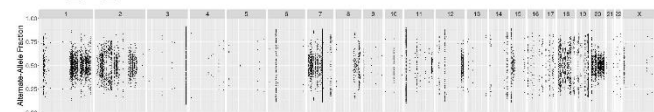
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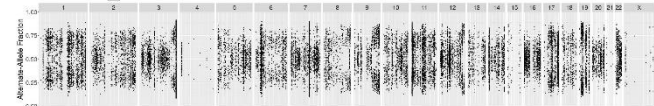
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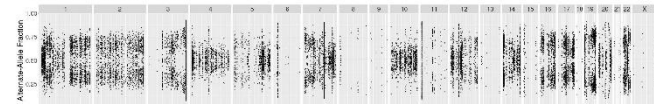
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MOCHA_33-M-E



MOCHA_27-L-E



Supplementary Figure: Whole exome sequencing of a selected number of organoids revealed significant copy number variation

References

1. Derouet MF, Allen J, Wilson GW et al. Towards personalized induction therapy for esophageal adenocarcinoma: organoids derived from endoscopic biopsy recapitulate the pre-treatment tumour. *Sci Rep* 2020;10(1):14514.