Utilisation of semiconductor sequencing for detection of actionable fusions in solid tumours

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Abstract:

Oncogenic fusions represent compelling druggable targets in solid tumours highlighted by the recent site agnostic FDA approval of larotrectinib for NTRK rearrangements. However screening for fusions in routinely processed tissue samples is constrained due to degradation of nucleic acid as a result of formalin fixation. To investigate the clinical utility of semiconductor sequencing optimised for detection of actionable fusion transcripts in formalin fixed samples, we have undertaken an analysis of test trending data generated by a clinically validated next generation sequencing platform designed to capture 867 of the most clinically relevant druggable driver-partner oncogenic fusions. Here we show across a real-life cohort of 1112 patients with solid tumours that actionable fusions occur at high frequency (7.4%) with

linkage to a wide range of targeted therapy protocols including seven fusion-drug matches with FDA/EMA approval and/or NCCN/ESMO recommendations and 80 clinical trials. The majority of actionable fusions identified were independent of tumour type in keeping with signalling via evolutionary conserved Wnt/ β -catenin, RAS/RAF/MEK/ERK, PI3K/AKT/MTOR, PLC γ /PKC and JAK/STAT pathways. Taken together our data indicates that semiconductor sequencing for detection of actionable fusions can be integrated into routine diagnostic pathology workflows enabling the identification of personalised treatment options that have potential to improve clinical cancer management across many tumour types.

Introduction:

Oncogenic fusion genes are an important class of driver mutation in solid tumors and hematological malignancies [1]. The rationale for targeting gene fusions was first highlighted by the significant clinical responses to imatinib in patients with BCR-ABL-positive chronic myeloid leukemia [2]. This therapeutic approach has now been extended to solid tumours, for example the targeting of ALK, ROS1, NTRK and RET fusion genes in non-small cell lung cancer (NSCLC) where the clinical impact has been far reaching with markedly improved survival outcomes [3, 4].

For decades, the major pathway for development and approval of oncology drugs has centred on histopathological parameters, anatomical location and clinical data from all-comers clinical trials [5]. However, the ecosystem for drug development has now changed dramatically with the recent landmark FDA approval for larotrectinib targeting neurotrophic receptor tyrosine kinase (NTRK) oncogenic fusions and pembrolizumab for tumours exhibiting microsatellite instability (MSI) and/or mismatch repair (MMR) defects. These drugs have been approved based on efficacy linked to specific molecular aberrations and not on conventional clinicopathological parameters [6, 7]. This new functional tissue agnostic approach to targeted therapies and immunotherapies is being rapidly accelerated by the expansion of molecular

basket clinical trials in which detection of an actionable genetic variant is used as the determinant for entry into a clinical trial [8, 9].

Although many oncogenic fusion genes have now been identified, few are screened as potential therapeutic targets in routine clinical practice. Testing is mostly restricted to the detection of ALK and ROS rearrangements in NSCLC [10]. This relates to the fact that fusion gene analysis is a particular challenge in the clinical context due to the complex combination and numbers of driver genes and partner genes involved in chromosomal rearrangements [1, 11-14]. Moreover the nucleic acid templates extracted from routine formalin fixed paraffin wax embedded tissues (FFPE) biopsy samples are characterised by low DNA/RNA yields with poor integrity and quality which is difficult to sequence particularly in relation to RNA fusion transcripts. Indeed the challenges of genomic profiling of FFPE clinical samples was recently highlighted in the summary report of the Genomic England 100,000 genomes project which utilized fluorescent based sequencing and concluded that analysis of FFPE samples for personalised medicine was infeasible [15].

The increasing use of targeted agents offers the great advantage of increased specificity and reduced toxicity when compared with conventional chemotherapy [16]. Meta-analysis in diverse tumor types has shown that a personalized strategy of treatment is an independent predictor of better outcomes and fewer toxicity associated deaths when compared with chemotherapy [17]. The recent advances in targeted next-generation sequencing (NGS) technologies optimized for nucleic acid templates extracted from FFPE tumour samples now provides the opportunity to conduct precision oncology testing as part of the routine diagnostic work flow. To investigate the potential role of clinically directed semiconductor sequencing in solid tumours we have established a clinically validated NGS platform optimised for analysis of FFPE clinical biopsy samples. This platform enables capture of 764 lead anti-cancer targeted agents/combinations and immunotherapy opportunities via analysis of actionable variants distributed across 505 genes. This screen includes detection of 867 druggable driver-partner oncogenic fusions via

analysis of 51 driver and 349 partner genes, with linkage to 140 targeted therapy protocols. All variants detected are “actionable” and therefore treatable by targeted therapies either on-market FDA and EMA approved, carrying ESMO and NCCN guideline references or currently in clinical trials, phases I-IV, worldwide [18].

Here we have undertaken a retrospective analysis of test trending data to investigate the types and frequency of clinically relevant fusions in solid tumours. Here we show that semiconductor sequencing can be incorporated into routine pathology diagnostic work flows enabling detection of druggable fusions at high frequency across many solid tumour types.

Materials and Methods

Patient demographics:

A retrospective analysis was performed on the trending data generated as part of routine comprehensive precision oncology NGS testing for solid tumours and collected in compliance with ISO15189:2012 requirements for monitoring of quality indicators. The research conducted in this study was limited to secondary use of information previously collected in the course of normal care (without an intention to use it for research at the time of collection) and therefore does not require REC review. The patients and service users were not identifiable to the research team carrying out trend data analysis. This is also in accordance with guidance issued by the National Research Ethics Service, Health Research Authority, NHS and follows the tenants of the Declaration of Helsinki. The trending data relates to a real-life cohort of 1112 patients tested between January 2018 and July 2019. The cancer types and histological classification of the study cohort are shown in S1 Table.

Comprehensive NGS genomic profiling

The NGS platform utilized for clinical testing is validated for clinical use and accredited by CLIA (ID 99D2170813) and by UKAS (9376) in compliance with ISO15189:2012 and following the guidelines published by the Association for Molecular Pathology and College of American Pathologists and IQN-Path ASBL as described [8, 19]. The performance characteristics are shown in S2 Table. The NGS platform includes the targeting of 51 driver genes and 349 partner genes enabling detection of 867 druggable driver-partner oncogenic fusions encompassing 400 unique genes and linked to 140 targeted therapy protocols. Genomic regions selected for analysis of clinically relevant fusions are shown in S3 Table.

RNA extraction, library preparation and sequencing:

RNA was extracted from FFPE curls cut at 10µm or from 5µm sections mounted onto unstained glass slides using the RecoverAll extraction kit (Ambion, Cat no.A26069). RNA samples were diluted to 5ng/µl and reverse transcribed to cDNA in a 96 well plate using the Superscript Vilo cDNA synthesis kit (CAT 11754250). Library construction, template preparation, template enrichment and sequencing were performed using Ion Ampliseq library 2.0 (Cat:4480441) and the Ion 540TM OT2 kit (Cat: A27753) according to the manufacturer's instructions. Sequencing was performed using the Ion S5 system 20 (Cat: A27212) utilising Ion 540TM chips (Cat:30 A27766).

Quality control

Sequencing runs were quality controlled using the following parameters according to manufacturer's instructions (Ion Reporter™ 5.10.1.0): chip loading >60% with >45 million reads observed, enrichment 98-100%, polyclonal percentage <55%, low quality <26%, usable reads > 30% and aligned bases were ≥80%,

unaligned bases were <20%, mean raw accuracy was >99% and overall read length between 100-115bp, average base coverage depth >1200, uniformity of amplicon (base) coverage >90%, amplicons were required to have less than 90% strand bias with >80% of amplicons reading end to end, on-target reads >85% and target base coverage at 1x, 20x, 100x and 500x >90% (S4 Table).

Data analysis:

Sequence alignment and variant calling was performed on The Torrent Suite™ Software (5.8.0). Alignment in Torrent Suite™ Software was performed using TMAP. The output BAM file was uploaded via the Ion Reporter Uploader plugin (5.8.32-1) to The Ion Reporter™ Software (5.10.1.0). Gene fusions were reported when occurring in >40 counts and meeting the threshold of assay specific internal RNA quality control with a sensitivity of 99% and PPV of 99%. Six internal expression quality controls were spiked into each sample to monitor assay performance with an acceptance cut-off of >15 reads in 5 out of 6 controls [Ion Reporter™ 5.10.1.0; default fusion view 5.10] (S3 Table). The results of variant annotation were organized hierarchically by gene, alteration, indication and level of evidence in relation to clinical actionability following the joint recommendation of the association of the AMP/ASCO/CAP [8, 9]. Tertiary analysis software was used to link variants to curated lists of relevant labels, guidelines, and global clinical trials [Oncomine™ Reporter (Cat:A34298)]; GlobalData clinical trials database].

Results:

Eighty nine actionable fusion gene events were identified in 1112 samples of solid tumours (Fig 1 and S5 Table). Eighty two of the 1112 samples tested had at least one actionable fusion gene representing a frequency of 7.4%. across the study cohort (S1 Table). The frequency of the different gene fusions

detected is shown in Fig 1. TBL1XR1-PIK3CA, MET-MET, WHSC1L1-FGFR1, EGFR VIII, FGFR3-TACC3 and EGFR-SEPT14 were identified as the most common druggable fusion events (Fig 1). Seven of the samples harboured two fusion genes. Four of these seven cases relate to glioblastoma in which fusion pairs CAPZA2-MET and FIP1L1-PDGFR α , CAPZA2-MET and MET-MET, EGFR VIII and PTPRZ1-MET, PTPRZ1-MET and TBL1XR1-PIK3CA were identified. Fusion pairs were also identified in colon (CAPZA2-MET and MET-MET), lung (PIK3CA-TBL1XR1 and RET-NCOA4) and pancreatic (FNDC3B-PIK3CA and TBL1XR1-PIK3CA) cancers (S4 Table).

The four most common fusions TBL1XR1-PIK3CA, MET-MET, WHSC1L1-FGFR1 and EGFR VIII representing 62% of all fusions were detected in a broad range of tumour types (Fig 2 and S4 Table). In contrast, some of the low frequency fusions, CCDC6-RET in thyroid and NSCLC lung cancer, FGFR2-BICC1 in cholangiocarcinoma, PTPRZ1-MET and KIF5B-RET in non-small cell lung (NSCLC) cancer (≤ 5 fusions detected) did show linkage to tumour type (Fig 1 and S5 Table). In tumour types with significant sample size ($n > 30$), a high frequency ($> 7\%$) of actionable fusions was observed in glioblastoma, head and neck cancers, cancers of unknown primary (CUP), prostate and pancreatic cancers and in NSCLC (S6 Table). Glioblastoma, colorectal, lung and breast cancers harboured the most diverse set of actionable fusion genes (Fig 3).

Actionable fusions can be targeted directly by small molecule inhibitors or alternatively through targeting of their cognate downstream signalling pathways. Bioinformatics pathway analysis showed the actionable fusions identified in this study were linked to a number of evolutionary conserved druggable cell signalling pathways including RAS/RAF/MEK/ERK, PIK3/AKT/MTOR, PLC γ /PKC, JAK/STAT and WNT/ β catenin (Table 1). Detected fusions were bioinformatically linked to a total of 73 targeted therapy protocols. These included seven fusion-drug matches with FDA/EMA approval and/or NCCN/ESMO recommendations (Table 1) either in indication or approved in “cancer of other type” and therefore meeting the tier criteria I and II level of clinical significance as defined by the Joint Consensus Recommendation of the Association

for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists [8]. These fusions were additionally linked to a total of 80 clinical trials investigating the efficacy of drugs either targeting the kinase fusion directly or alternatively using inhibitors targeting pathways downstream (Table 1).

Table 1. Actionable fusion genes in solid tumours.

<i>Genomic alteration</i>	<i>Cancer type</i>	<i>Pathway</i>	<i>Relevant therapies</i>	<i>Clinical trials</i>	<i>Relevant Therapy</i>
			<i>*approval in other cancer type</i>		
<i>KANK1 - NTRK3</i>	Breast	RAS/RAF/MEK/E RK	entrectinib	NCT02576431	larotrectinib
		PI3K/AKT/MTOR pathway	larotrectinib	NCT02637687	larotrectinib
		PLCy/PKC		NCT02568267	entrectinib
			*Larotrectinib (all solid tumours)	NCT02465060	larotrectinib
				NCT03155620	larotrectinib
				NCT02920996	merestinib
				NCT03213704	larotrectinib
				NCT03297606	temsirolimus
				No NCT ID	entrectinib
				NCT03093116	repotrectinib
<i>FGFR1 - NRG1</i>	Breast	RAS/RAF/MEK/E RK		NCT03215511	LOXO-195
		PI3K/AKT/MTOR pathway		NCT02052778	TAS-120
				NCT02393248	chemotherapy, INCMGA00012, pembrolizumab, pemigatinib, trastuzumab
		PLCy/PKC		NCT01948297	FF-284
		JAK/STAT		NCT03834220	FF-284
				NCT02272998	ponatinib
				NCT03297606	sunitinib
				NCT03160833	HMPL-453
				NCT03929965	anlotinib hydrochloride
				NCT03235570	pemigatinib
<i>TMEM178B - MET</i>	Colorectal	RAS/RAF/MEK/E RK		NCT03297606	crizotinib
		PI3K/AKT		NCT03175224	bozitinib
				NCT02219711	sitravatinib
<i>EGFR - SEPT14</i>	Colorectal, Glioblastoma	PI3K/AKT/MTOR pathway		No NCT ID	cetuximab + chemotherapy
		RAS/RAF/MEK/E RK		No NCT ID	cetuximab + chemotherapy

<i>CAPZA2 - MET</i>	Glioblastoma, Colorectal	RAS/RAF/MEK/E RK	NCT03152435	CART-EGFR
			NCT03454620	GC1118A + chemotherapy
			NCT03319459	cetuximab + FATE-NK100
			NCT03297606	erlotinib
			NCT02013089	erlotinib, gefitinib
			NCT02423525	afatinib
			NCT03598244	volitinib
			PI3K/AKT	NCT03297606
				crizotinib
			NCT03175224	bozitinib
<i>FIP1L1 - PDGFRA</i>	Glioblastoma	RAS/RAF/MEK/E RK	NCT02219711	sitravatinib
			NCT03352427	dasatinib, everolimus
			PI3K/AKT/MTOR pathway	
<i>PTPRZ1 - MET</i>	Glioblastoma	RAS/RAF/MEK/E RK	NCT03598244	volitinib
			NCT03297606	crizotinib
			NCT03175224	bozitinib
			NCT02219711	sitravatinib
<i>AGK - BRAF</i>	Glioblastoma	RAS/RAF/MEK/E RK	NCT02639546	cobimetinib
			NCT02029001	sorafenib
			NCT03843775	binimetinib + encorafenib
			NCT03520075	ASTX029
			NCT03905148	lifirafenib, PD-0325901
			NCT03415126	ASN007
			NCT02857270	abemaciclib, cetuximab, chemotherapy, encorafenib, LY3214996, midazolam
			NCT02607813	LXH254
			NCT03634982	RMC-4630
			NCT03292250	alpelisib
<i>TBL1XR1 - PIK3CA</i>	Breast, Cervical, Colorectal, CUP, endometrial, gastric, glioblastoma, head and neck, GOJ, NSCLC, Oesophageal, Ovarian, Pancreatic, Thyroid, Vulva	PI3K/AKT/MTOR pathway	NCT03065062	gedatolisib + palbociclib
			NCT03297606	temsirolimus

<i>FGFR3 - TACC3</i>	Glioblastoma, Head and Neck and prostate	RAS/RAF/MEK/E RK	*erdafitinib (Bladder cancer)	NCT02576444	capivasertib, olaparib
				NCT03673787	atezolizumab + ipatasertib
				NCT03805399	chemotherapy, mTOR inhibitor
				NCT02615730	GSK-2636771 + chemotherapy
				NCT03675893	abemaciclib + LY-3023414
				NCT03292250	nintedanib
				NCT01948297	FF-284
				NCT03834220	FF-284
				NCT02272998	ponatinib
				NCT03297606	sunitinib
<i>WHSC1L1 - FGFR1</i>	Breast Cancer, CUP, Renal Cell Carcinoma, Oesophageal, Ovarian, Sarcoma, Small bowel	RAS/RAF/MEK/E RK		NCT02052778	TAS-120
				NCT03160833	HMPL-453
				NCT03929965	anlotinib hydrochloride
				NCT03235570	pemigatinib
				NCT02872714	pemigatinib
				NCT02052778	TAS-120
				NCT03160833	HMPL-453
				NCT03834220	FF-284
				NCT02272998	ponatinib
				NCT03297606	sunitinib
<i>FGFR2 - BICC1</i>	Cholangiocarcinoma (Liver)	RAS/RAF/MEK/E RK	*erdafitinib (Bladder cancer)	NCT01948297	FF-284
				NCT03929965	anlotinib hydrochloride
				NCT03235570	pemigatinib
				NCT02393248	chemotherapy, INCMGA00012, pembrolizumab, pemigatinib, trastuzumab
				NCT03822117	pemigatinib
				NCT02052778	futibatinib
				NCT03230318	derazantinib
				NCT03773302	infigratinib
				NCT03656536	pemigatinib
				NCT02699606	erdafitinib
				NCT03834220	FF-284

<i>KIF5B - RET</i>	NSCLC	PI3K/AKT/MT OR RAS/RAF/MEK/E RK PLCy/PKC	Cabozantinib vandetanib	NCT02150967	infigratinib
				NCT02924376	pemigatinib
				NCT02691793	sunitinib
				NCT02272998	ponatinib
				NCT03297606	sunitinib
				NCT02052778	TAS-120
				NCT02393248	chemotherapy, INCMGA00012, pembrolizumab, pemigatinib, trastuzumab
				NCT03160833	HMPL-453
				NCT01948297	FF-284
				NCT03929965	anlotinib hydrochloride
				NCT03235570	pemigatinib
				No NCT ID	alectinib
				NCT03194893	alectinib, crizotinib
				NCT03391869	ipilimumab, nivolumab, radiation therapy, surgical intervention
				NCT01639508	cabozantinib
				NCT02314481	alectinib
				NCT03445000	alectinib
				NCT02540824	apatinib
				NCT02699606	erdafitinib
				NCT02299141	nintedanib
				NCT02664935	sitravatinib
				No NCT ID	targeted therapy, targeted therapy + chemotherapy
				NCT03157128	LOXO-292
				NCT03037385	BLU-667
				NCT03780517	BOS172738
<i>SND1 - MET</i>	NSCLC	RAS/RAF/MEK/E RK PI3K/AKT		NCT02219711	sitravatinib
				NCT02029001	sorafenib
				NCT02450123	sunitinib
				NCT02691793	sunitinib
				NCT02272998	ponatinib
				NCT03297606	sunitinib
				NCT03088930	crizotinib
				NCT02323126	capmatinib + nivolumab
				NCT02414139	capmatinib
				NCT02219711	sitravatinib
<i>RET - NCOA4</i>	NSCLC	PI3K/AKT/MT OR	cabozantinib	NCT03297606	crizotinib
				NCT03175224	bozitinib
				No NCT ID	alectinib

CCDC6 - RET	RAS/RAF/MEK/E RK PLCy/PKC		vandetanib	NCT03194893	alectinib, crizotinib
				NCT03391869	ipilimumab, nivolumab, radiation therapy, surgical intervention
				NCT02314481	alectinib
				NCT03445000	alectinib
				NCT02540824	apatinib
				NCT01639508	cabozantinib
				NCT02699606	erdafitinib
				NCT02299141	nintedanib
				NCT02664935	sitravatinib
				No NCT ID	targeted therapy, targeted therapy + chemotherapy
				NCT03157128	LOXO-292
				NCT03037385	BLU-667
				NCT03780517	BOS172738
				NCT02219711	sitravatinib
				NCT02029001	sorafenib
				NCT02450123	sunitinib
				NCT02691793	sunitinib
				NCT02272998	ponatinib
				NCT03297606	sunitinib
	NSCLC, Thyroid	PI3K/AKT/M TOR	cabozantinib (NSCLC)	No NCT ID	alectinib
		RAS/RAF/MEK/E RK PLCy/PKC	vandetanib (NSCLC)	NCT03194893	alectinib, crizotinib
				NCT03391869	ipilimumab, nivolumab, radiation therapy, surgical intervention
				NCT02314481	alectinib
				NCT03445000	alectinib
				NCT02540824	apatinib
				NCT01639508	cabozantinib
				NCT02699606	erdafitinib
				NCT02299141	nintedanib
				NCT02664935	sitravatinib
				No NCT ID	targeted therapy, targeted therapy + chemotherapy
				NCT03157128	LOXO-292
				NCT03037385	BLU-667
				NCT03780517	BOS172738
				NCT02219711	sitravatinib
				NCT02029001	sorafenib
				NCT02450123	sunitinib
				NCT02691793	sunitinib
				NCT02272998	ponatinib

<i>MET - MET</i>	Bladder, CUP, Colorectal, Glioblastoma, NSCLC, Pancreatic cancer, Squamous cell carcinoma, Renal cell carcinoma	RAS/RAF/MEK/E RK	crizotinib (NSCLC)	NCT03297606	sunitinib
				NCT01945762	vandetanib
				NCT03297606	crizotinib
		PI3K/AKT/MT OR		NCT03175224	bozitinib
				NCT02219711	sitravatinib
				NCT03088930	crizotinib
				NCT03729297	cabozantinib
				NCT02323126	capmatinib + nivolumab
				NCT02213289	rilotumumab + chemotherapy
				NCT02414139	capmatinib
				NCT03598244	volitinib
				NCT02867592	cabozantinib
				NCT02465060	crizotinib
				NCT03911193	cabozantinib
				NCT02867592	cabozantinib
				NCT02465060	crizotinib
				NCT02029001	sorafenib
<i>TMEM178B - BRAF</i>	Teratoma (Other)	RAS/RAF/MEK/E RK		NCT03843775	binimetinib + encorafenib
				NCT03520075	ASTX029
				NCT02639546	cobimetinib
				NCT03905148	lifirafenib, PD-0325901
				NCT03415126	ASN007

<i>FNDC3B - PIK3CA</i>	Pancreas	PI3K/AKT/MTOR pathway	NCT02857270	abemaciclib, cetuximab, chemotherapy, encorafenib, LY3214996, midazolam
			NCT02607813	LXH254
			NCT03634982	RMC-4630
			NCT02013089	sorafenib, sunitinib
			NCT03714958	HDM-201 + trametinib
			NCT03065062	gedatolisib + palbociclib
			NCT03297606	temsirolimus
			NCT02576444	capiasertib, olaparib
			NCT03673787	atezolizumab + ipatasertib
			NCT02029001	sorafenib
<i>SND1 - BRAF</i>	Prostate	RAS/RAF/MEK/E RK	NCT03843775	binimetinib + encorafenib
			NCT03520075	ASTX029
			NCT02639546	cobimetinib
			NCT03905148	lifirafenib, PD-0325901
			NCT03415126	ASN007
			NCT02857270	abemaciclib, cetuximab, chemotherapy, encorafenib, LY3214996, midazolam
			NCT02607813	LXH254
			NCT03634982	RMC-4630
			NCT02029001	sorafenib
			NCT03843775	binimetinib + encorafenib
<i>BRAF - MRPS33</i>	Prostate	RAS/RAF/MEK/E RK	NCT03520075	ASTX029
			NCT02639546	cobimetinib
			NCT03905148	lifirafenib, PD-0325901
			NCT03415126	ASN007
			NCT02857270	abemaciclib, cetuximab, chemotherapy, encorafenib, LY3214996, midazolam
			NCT02607813	LXH254
			NCT03634982	RMC-4630
			NCT03297606	erlotinib
			NCT02423525	afatinib
			NCT03319459	cetuximab + FATE-NK100
<i>EGFR - EGFR</i>	Glioblastoma, Prostate	PI3K/AKT/MTOR pathway	NCT03297606	erlotinib
			NCT02423525	afatinib
			NCT03319459	cetuximab + FATE-NK100
			NCT03319459	cetuximab + FATE-NK100

<i>PCM1 - BRAF</i>	Sarcoma	RAS/RAF/MEK/E RK	NCT02639546	cobimetinib
			NCT02029001	sorafenib
			NCT03843775	binimetinib + encorafenib
			NCT03520075	ASTX029
			NCT03905148	lifirafenib, PD-0325901
			NCT03415126	ASN007
			NCT02857270	abemaciclib, cetuximab, chemotherapy, encorafenib, LY3214996, midazolam
			NCT02607813	LXH254
			NCT03634982	RMC-4630
<i>PTPRK - RSPO3</i>	Colorectal	WNT/ β catenin	NCT01351103	LGK974
<i>EIF3E - RSPO2</i>	Colorectal	WNT/ β catenin	NCT01351103	LGK974

Fig 1. Actionable fusion gene landscape in solid tumours.

Fig 2. Frequency of the four most common actionable gene fusions in solid tumours.

Fig 3. Frequency of actionable gene fusions in glioblastoma, colorectal, NSCLC and breast cancers.

Discussion:

Advances in somatic cancer genetics and genomic profiling is leading to a shift in treatment paradigm from relatively non-specific empirically directed cytotoxic chemotherapies to a more biologically informed targeted approach [20-22]. The drug-target pairing that links a dysregulated molecular pathway with a cognate therapeutic agent defines the modern era of precision oncology. Targeted agents and immunotherapies are associated with superior response rates, fewer side effects and reduced healthcare costs when compared with nonselective chemotherapy [16, 17, 23].

Analysis of our NGS test trending data has shown that actionable fusions occur across a wide range of tumour types providing more personalised treatment options to cancer patients. Actionable fusions were identified at high frequency, 7.3% across all solid tumour types, rising to 23% in the case of glioblastoma. Notably the majority of high frequency fusions were agnostic of tumour type. A proportion of low

frequency fusions did however exhibit tumour type specificity including CCDC6-RET fusions in thyroid and lung cancer, FGFR2-BICC1 in cholangiocarcinoma, PTPRZ1-MET in glioblastoma, EIF3E-RSPO2 and PTPRK-RSPO3 in colorectal cancer and KIF5B-RET in NSCLC [24-28]. Other low frequency fusions namely, AGK-BRAF, FIP1L1-PDGFR, FNDC3B-PIK3CA, RET-NCOA4, SND1-BRAF, TMEM178B-MET, TMEM178B-BRAF, KANK1-NTRK3, EIF3E-RSPO2 and PTPRK-RSPO3 have been previously reported as rare fusions in tumours of other type [27, 29-35]. Non targeted novel fusions were also identified including FGFR1-NRG1 in breast, BRAF-MRPS33 in prostate, SND1-MET in lung, PCM1-BRAF in sarcoma and TMEM178B-MET in rectal cancer.

Detected actionable fusions were found to interact with one or more of the major evolutionary conserved cell signalling pathways, namely RAS/RAF/MEK/ERK, PI3K/AKT/MTOR, PLC γ /PKC, JAK/STAT and WNT/ β catenin. These are key signalling networks mediating fundamental processes including cell proliferation, differentiation, cell migration and apoptosis which are involved in homeostasis across all tissue types [36]. This may account for the fact that the majority of detected actionable fusions are independent of tumour type or tissue of origin. The minority population of tumour specific fusions identified also involve interaction with these conserved signalling networks suggesting that the tumour specificity of these particular fusions does not relate to tissue specific signalling pathways. Moreover tissue specificity does not appear to be determined by tissue specific expression of the partner or driver genes involved in these fusions. For example, in the case of KIF5B-RET in NSCLC, KIF5B is expressed across a broad range of tissue types in keeping with its ubiquitous function as key component of the mitotic machinery and similarly RET is expressed across a broad range of tissue types [37, 38]. The mechanisms relating to tissue specificity remain poorly defined but may relate to other factors, for example interphase gene proximity (spatial proximity) that can facilitate generation of fusion genes [11, 39, 40]. Interestingly some fusions were associated with a particular tumour subtype, for example all fusions detected in breast cancer were

restricted to cancers of ductal type and in lung cancers to those of NSCLC type. Cellular morphology has been postulated to represent a holistic readout of the complex genomic and gene expression changes in cancer cells and it is interesting to speculate that activation of the cognate signalling pathways linked to these particular fusions drive genomic changes and transcriptome profiles that act as determinants of these specific morpho-phenotypes [41].

The majority of detected druggable oncogenic driver genes represent tyrosine kinases (TKs) with partner genes encoding coil coiled domains leading to ligand-independent homodimerization, TK activation and dysregulated growth. However, it is also possible that disruption of the partner gene itself may also contribute to tumourigenesis through putative tumour suppressor roles. A number of the partner genes identified including TACC, CCDC6, EIF3E and KIF5B participate in DNA damage repair or mitotic chromosomal segregation [42-44]. Dysregulation of these genes have potential to drive error-prone DNA replication leading to genomic instability. This is in keeping with the notion that gene fusion events may function as a “two hit” model for multistep tumourigenesis.

Currently routine clinical molecular testing in relation to druggable fusion genes is limited to NSCLC, but even in this tumour type, analysis covers only a small number of potential driver and partner genes, namely ALK and ROS rearrangements [10]. Here we have shown that semiconductor sequencing analysis of fusion transcripts in routine FFPE samples applied across all solid tumour types enables comprehensive analysis of hundreds of druggable fusion genes. Their detection enables clinical evidence based linkage to a broad therapeutic armamentarium of targeted therapies which can have a major impact in the improved clinical management of advanced solid tumours. The majority of targeted agents are directed against the fusions themselves but alternatively treatment protocols also include targeting components of the cognate signalling pathways downstream. For example, the TBL1XR1-PIK3CA fusion is linked to targeted

therapies inhibiting PI3K directly (alpelisib) but also inhibitors targeting downstream signalling components including AKT (capivasertib), MTOR temsirolimus) and CDK4/6 (abemaciclib). Although a targeted agent for TRK fusions now has FDA approval, this rearrangement was identified as a rare event in our cohort of solid tumours (<1%) in keeping with previous reports [1]. In contrast here we have shown that targeting high frequency fusions such as TBL1XR1-PIK3CA, MET-MET, WHSC1L1-FGFR1 fusions offer much broader clinical utility across all tumour types.

In summary we have shown that NGS semiconductor sequencing optimised for analysis of druggable fusions occur at high frequency in solid tumours and that their coverage is therefore an important component of comprehensive precision oncology profiling, thereby ensuring that all targeted therapeutic opportunities are captured. Notably, the majority of actionable fusions are not tumor type specific reinforcing the “site agnostic” approach to genomic profiling and supporting the concept of “molecular basket” clinical trials. Importantly we have also demonstrated that adoption of semiconductor sequencing methodologies enables comprehensive precision oncology profiling to be applied robustly to routine FFPE clinical biopsy samples allowing integration with globally established routine diagnostic pathology workflows.

Author contributions

ML and GW were responsible for study design, conceptualization, supervision and manuscript preparation. RT, TH and KH performed the data analysis and visualization.

Data availability

The datasets analysed during the current study are provided in the Supplementary File. Further technical details are available from the corresponding author upon reasonable request.

Ethical statement

The research conducted in this study was limited to secondary use of information previously collected in the course of normal care (without an intention to use it for research at the time of collection) and therefore does not require REC review. The patients and service users were not identifiable to the research team carrying out trend data analysis. This is also in accordance with guidance issued by the National Research Ethics Service, Health Research Authority, NHS and follows the tenants of the Declaration of Helsinki.

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REFERENCES

1. Stransky N, Cerami E, Schalm S, Kim JL, Lengauer C. The landscape of kinase fusions in cancer. Nat Commun. 2014;5: 4846.
2. Druker BJ, Talpaz M, Resta DJ, Peng B, Buchdunger E, Ford JM, et al. Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. N Engl J Med. 2001;344(14): 1031-1037.
3. Ferrara R, Auger N, Auclin E, Besse B. Clinical and Translational Implications of RET Rearrangements in Non-Small Cell Lung Cancer. J Thorac Oncol. 2018;13(1): 27-45.
4. Cheng TY, Cramb SM, Baade PD, Youlten DR, Nwogu C, Reid ME. The International Epidemiology of Lung Cancer: Latest Trends, Disparities, and Tumor Characteristics. J Thorac Oncol. 2016;11(10): 1653-1671.
5. Jørgensen JT. A paradigm shift in biomarker guided oncology drug development. Ann Transl Med. 2019;7(7): 148.
6. Scott LJ. Larotrectinib: First Global Approval. Drugs. 2019;79(2): 201-206.
7. Lemery S, Keegan P, Pazdur R. First FDA Approval Agnostic of Cancer Site - When a Biomarker Defines the Indication. N Engl J Med. 2017;377(15): 1409-1412.
8. Li MM, Datto M, Duncavage EJ, Kulkarni S, Lindeman NI, Roy S, et al. Standards and Guidelines for the Interpretation and Reporting of Sequence Variants in Cancer: A Joint Consensus Recommendation of the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists. J Mol Diagn. 2017;19(1): 4-23.

9. Mateo J, Chakravarty D, Dienstmann R, Jezdic S, Gonzalez-Perez A, Lopez-Bigas N, et al. A framework to rank genomic alterations as targets for cancer precision medicine: the ESMO Scale for Clinical Actionability of molecular Targets (ESCAT). *Ann Oncol.* 2018;29(9): 1895-1902.
10. Garinet S, Laurent-Puig P, Blons H, Oudart JB. Current and Future Molecular Testing in NSCLC, What Can We Expect from New Sequencing Technologies?. *J Clin Med.* 2018;7(6): 144.
11. Tuna M, Amos CI, Mills GB. Molecular mechanisms and pathobiology of oncogenic fusion transcripts in epithelial tumors. *Oncotarget.* 2019;10(21): 2095-2111.
12. Gao Q, Liang WW, Foltz SM, Mutharasu G, Jayasinghe RG, Cao S, et al. Driver Fusions and Their Implications in the Development and Treatment of Human Cancers. *Cell Rep.* 2018;23(1): 227-238.e3.
13. Barr FG. Fusion genes in solid tumors: the possibilities and the pitfalls. *Expert Rev Mol Diagn.* 2016;16(9): 921-923.
14. Latysheva NS, Babu MM. Discovering and understanding oncogenic gene fusions through data intensive computational approaches. *Nucleic Acids Res.* 2016;44(10): 4487-4503.
15. Turnbull C. Introducing whole-genome sequencing into routine cancer care: the Genomics England 100 000 Genomes Project. *Ann Oncol.* 2018;29(4): 784-787.
16. Haslem DS, Chakravarty I, Fulde G, Gilbert H, Tudor BP, Lin K, et al. Precision oncology in advanced cancer patients improves overall survival with lower weekly healthcare costs. *Oncotarget.* 2018;2;9(15): 12316-12322.
17. Schwaederle M, Zhao M, Lee JJ, Eggermont AM, Schilsky RL, Mendelsohn J, et al. Impact of Precision Medicine in Diverse Cancers: A Meta-Analysis of Phase II Clinical Trials. *J Clin Oncol.* 2015; 10;33(32): 3817-25.

18. Loddo M, Hardisty KM, Thatcher RP, Haddow TE, Williams GH. The actionable genomic mutational landscape in solid tumours. *J Clin Oncol*. 2020;38(15): suppl.e13642.
19. Deans ZC, Costa JL, Cree I, Dequeker E, Edsjö A, Henderson S, et al. IQN Path ASBL. Integration of next-generation sequencing in clinical diagnostic molecular pathology laboratories for analysis of solid tumours; an expert opinion on behalf of IQN Path ASBL. *Virchows Arch*. 2017;470(1): 5-20.
20. Gaines ME. Charting a Course: Navigating the Hype and the Hope of Precision Medicine Together. ASCO Guest commentary. Available at <https://connection.asco.org/blogs/charting-course-navigating-hype-and-hope-precision-medicine-together>. Deposited 5 August, 2019.
21. Marquart J, Chen EY, Prasad V. Estimation of the Percentage of US Patients With Cancer Who Benefit From Genome-Driven Oncology. *JAMA Oncol*. 2018;4(8): 1093-1098.
22. Tsimberidou AM, Hong DS, Ye Y, Cartwright C, Wheeler JJ, Falchook GS, et al. Initiative for Molecular Profiling and Advanced Cancer Therapy (IMPACT): An MD Anderson Precision Medicine Study. *JCO Precis Oncol*. 2017;2017.
23. Gong J, Pan K, Fakih M, Pal S, Salgia R. Value-based genomics. *Oncotarget*. 2018;9(21): 15792-15815.
24. Okamoto K, Kodama K, Takase K, Sugi NH, Yamamoto Y, Iwata M, et al. Antitumor activities of the targeted multi-tyrosine kinase inhibitor lenvatinib (E7080) against RET gene fusion-driven tumor models. *Cancer Lett*. 2013;340(1): 97-103.
25. Ying X, Tu J, Wang W, Li X, Xu C, Ji J. FGFR2-BICC1: A Subtype Of FGFR2 Oncogenic Fusion Variant In Cholangiocarcinoma And The Response To Sorafenib. *Onco Targets Ther*. 2019;12: 9303-9307.
26. Bao ZS, Chen HM, Yang MY, Zhang C-B, Yu K, Ye W-L, et al. RNA-seq of 272 gliomas revealed a novel, recurrent PTPRZ1-MET fusion transcript in secondary glioblastomas. *Genome Res*. 2014;24(11): 1765-1773.

27. Seshagiri S, Stawiski EW, Durinck S, Modrusan Z, Storm EE, Conboy CB, et al. Recurrent R-spondin fusions in colon cancer. *Nature*. 2012;488(7413): 660-664.
28. Ferrara R, Auger N, Auclin E, Besse B. Clinical and Translational Implications of RET Rearrangements in Non-Small Cell Lung Cancer. *J Thorac Oncol*. 2018;13(1): 27-45.
29. Sisdelli L, Cordioli MICV, Vaisman F, Moraes L, Colozza-Gama GA, Alves PAG, et al. AGK-BRAF is associated with distant metastasis and younger age in pediatric papillary thyroid carcinoma. *Pediatr Blood Cancer*. 2019;66(7): e27707.
30. Ibata M, Iwasaki J, Fujioka Y, Nakagawa K, Darmanin S, Onozawaet M, et al. Leukemogenic kinase FIP1L1-PDGFRα and a small ubiquitin-like modifier E3 ligase, PIAS1, form a positive cross-talk through their enzymatic activities. *Cancer Sci*. 2017;108(2): 200-207.
31. Stransky N, Cerami E, Schalm S, Kim JL, Lengauer C. The landscape of kinase fusions in cancer. *Nat Commun*. 2014;5: 4846.
32. Kim SY, Oh SO, Kim K, Lee J, Kang S, Kim K-M, et al. NCOA4-RET fusion in colorectal cancer: Therapeutic challenge using patient-derived tumor cell lines. *J Cancer*. 2018;9(17): 3032-3037.
33. Jang JS, Lee A, Li J, Liyanage H, Yang Y, Guo L, et al. Common Oncogene Mutations and Novel SND1-BRAF Transcript Fusion in Lung Adenocarcinoma from Never Smokers. *Sci Rep*. 2015;5: 9755.
34. Kim HS, Jung M, Kang HN, Kim H, Park C-W, Kim S-M, et al. Oncogenic BRAF fusions in mucosal melanomas activate the MAPK pathway and are sensitive to MEK/PI3K inhibition or MEK/CDK4/6 inhibition. *Oncogene*. 2017;36(23): 3334-3345.
35. Catic A, Kurtovic-Kozaric A, Johnson SH, Vasmatazis G, Pins MR, Kogan J. A novel cytogenetic and molecular characterization of renal metanephric adenoma: Identification of partner genes involved in translocation t(9;15)(p24;q24). *Cancer Genet*. 2017;214-215: 9-15.

36. Weinberg RA. Cytoplasmic signalling circuitry programs; many of the traits of cancer. In The biology of cancer. 15th edition. Garland Science, Taylor & Francis group. 2007: 159-207.
37. Maliga Z, Junqueira M, Toyoda Y, Ettinger A, Mora-Bermúdez F, Klemm RW, et al. A genomic toolkit to investigate kinesin and myosin motor function in cells. Nat Cell Biol. 2013;15(3): 325-334.
38. RET proto-oncogene. Tissue atlas. The human protein atlas.
<https://www.proteinatlas.org/ENSG00000165731-RET/tissue>
39. Gandhi M, Medvedovic M, Stringer JR, Nikiforov YE. Interphase chromosome folding determines spatial proximity of genes participating in carcinogenic RET/PTC rearrangements. Oncogene. 2006;25(16): 2360-2366.
40. Nikiforova MN, Stringer JR, Blough R, Medvedovic M, Fagin JA, Nikiforov YE. Proximity of chromosomal loci that participate in radiation-induced rearrangements in human cells. Science. 2000;290(5489): 138-141.
41. Wu PH, Gilkes DM, Phillip JM, Narkar A, Cheng TW-T, Marchand J, et al. Single-cell morphology encodes metastatic potential. Sci Adv. 2020;6(4): eaaw6938.
42. Tang NH, Okada N, Fong CS, Arai K, Sato M, Toda T. Targeting Alp7/TACC to the spindle pole body is essential for mitotic spindle assembly in fission yeast. FEBS Lett. 2014;588(17): 2814-2821.
43. Merolla F, Pentimalli F, Pacelli R, Vecchio G, Fusco A, Grieco M, et al. Involvement of H4(D10S170) protein in ATM-dependent response to DNA damage. Oncogene. 2007;26(42): 6167-6175.
44. Morris C, Tomimatsu N, Richard DJ, Cluet D, Burma S, Khanna KK, et al. INT6/EIF3E interacts with ATM and is required for proper execution of the DNA damage response in human cells. Cancer Res. 2012;72(8): 2006-2016.

Figure 1. Actionable fusion gene landscape in solid tumours.

Figure 2. Frequency of the four most common actionable gene fusions in solid tumours.

Figure 3. Frequency of actionable gene fusions in glioblastoma, colorectal, NSCLC and breast cancers.