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Original Research

Pan-tumor survey of *RET* fusions as detected by next-generation RNA sequencing identified *RET* fusion positive colorectal carcinoma as a unique molecular subset

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ABSTRACT

Background: *RET* fusions are driver alterations in cancer and are most commonly found in non-small cell lung cancer and well-differentiated thyroid cancer. However, *RET* fusion have been reported in other solid tumors.
Material and methods: A retrospective analysis of *RET*+ solid malignancies identified by targeted RNA sequencing and whole transcriptome sequencing of clinical tumor samples performed at Caris Life Science (Phoenix, AZ).
Results: As of March 22, 2022, a total of 378 *RET*+ solid malignancies were identified in 15 different tumor types and carcinoma of unknown primary (CUP) that underwent next-generation RNA sequencing. *RET*+ NSCLC and *RET*+ thyroid cancer constituted 66.9% and 11.1% of the *RET*+ solid malignancies, respectively. *RET*+ colorectal adenocarcinoma and *RET*+ breast adenocarcinoma constituted 10.1% and 2.6%, respectively. The estimated frequency of *RET* fusions within specific tumor types were NSCLC 0.7%, thyroid cancer 3.1%, colorectal cancer 0.2% and breast cancer 0.1%. *KIF5B* (46.8%) was the most common fusion partner followed by *CCDC6* (28.3%) and *NCOA4* (13.8%) in *RET*+ solid tumors. *KIF5B-RET* was the dominant fusion variant in *RET*+ NSCLC, *NCOA4-RET* was the dominant variant in *RET*+ colorectal carcinoma, and *CCDC6-RET* was the dominant variant in thyroid cancer. The most common single gene alterations in *RET*+ tumors were *TP53* (34.8%), *RASA1* (14.3%) and *ARIAD1A* (11.6%). *RET*+ CRC had a high median TMB of 20.0 and were commonly MSI-H.
Conclusions: *RET* fusions were identified in multiple tumor types. With a higher median TMB and commonly MSI-H, *RET* fusion positive CRC may be a unique molecular subset of CRC.

Introduction

Recurrent fusions of receptor tyrosine kinase (RTK) genes yielding constitutively active chimeras have been recognized as key actionable driver mutations in diverse solid malignancies [1]. Among the 58 human RTKs [2], there are US Food and Drug Administration (FDA) approved treatment for anaplastic lymphoma kinase (ALK), c-ROS1 (ROS1), rearranged during transfection (RET), fibroblastic growth factor

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receptor (FGFR2–3), and neutrophin receptor tyrosine kinase (NTRK1–3) fusion positive tumors. However, most of the US FDA approvals are tumor histologic specific: ALK (non-small cell lung cancer [NSCLC]), ROS1 (NSCLC), and FGFR2–3 (urothelial, cholangiocarcinoma). NTRK1–3 fusions was the first to obtain tumor agnostic treatment approval due to the extremely rare incidence of NTRK fusions and FDA has recently expanded approval of selpercatinib to include *RET* fusion positive tumors outside of NSCLC and thyroid cancers.

While these RTK fusions can be found in many solid tumor types albeit in a lower frequency (i.e. ALKoma [3], REToma [4]), the main biology of the pathological process is universal and not tumor histology-specific. Especially as FDA has recently expanded its approval on selpercatinib to include adult patients with advanced solid tumors harboring *RET* fusions, it is important to identify RTK fusions systemically beyond the specific tumor histologic type and to expand the horizon of patients with RTK fusion positive cancer who may benefit from targeted treatment. We must also strive to raise awareness among clinicians, pharmaceutical companies and regulatory authorities to screen and enroll these patients in future clinical trials.

In this study, we performed a large-scale pan-tumor survey of *RET* fusions detected by next generation RNA sequencing to identify characterize the molecular characteristics of *RET*+ solid tumors.

Materials and methods

Patient cohort

An institutional review board (IRB)–approved, retrospective assessment of a deidentified molecular profiling database was surveyed for solid tumors that underwent RNA based tumor profiling. From a cohort including all cases submitted to a Clinical Laboratory Improvement Amendments (CLIA)–certified laboratory (Caris Life Sciences) for comprehensive genomic profiling, all unique cases that underwent successful fusion testing for targeted RNA sequencing were identified. This study was conducted in accordance with guidelines of the Declaration of Helsinki, Belmont report, and U.S. Common rule. In keeping with 45 CFR 46.101(b)(4), this study was performed utilizing retrospective, deidentified clinical data.

Fusion detection

RET fusion was detected by either the ArcherDx fusion assay (Archer FusionPlex Solid Tumor panel) or the Illumina NovaSeq platform (Illumina, Inc., San Diego, CA) with the use of the Agilent SureSelect Human All Exon V7 bait panel (Agilent Technologies, Santa Clara, CA). For the ArcherDx fusion assay, formalin-fixed paraffin-embedded tumor samples were microdissected to enrich the sample to ≥20% tumor nuclei, and mRNA was isolated and reverse transcribed into complementary DNA (cDNA). Unidirectional gene-specific primers were used to enrich for target regions, followed by Next-Generation sequencing (Illumina MiSeq platform). For fusion detection using the Illumina NovaSeq platform, FFPE specimens underwent pathology review to diagnose percent tumor content and tumor size; a minimum of 10% of tumor content in the area for microdissection was required to enable enrichment and extraction of tumor-specific RNA. Qiagen RNA FFPE tissue extraction kit was used for extraction, and the RNA quality and quantity was determined using the Agilent TapeStation.

Next-generation RNA sequencing

The whole transcriptome sequencing has previously been described. Briefly, next generation sequencing (NGS) was performed on genomic DNA isolated from formalin-fixed paraffin-embedded (FFPE) tumor samples using the NextSeq platform (Illumina, Inc., San Diego, CA). A custom-designed SureSelect XT assay was used to enrich 592 whole-gene targets (Agilent Technologies, Santa Clara, CA). All variants were detected with > 99% confidence based on allele frequency and amplicon coverage, with an average sequencing depth of coverage of > 500 and an analytic sensitivity of 5%. Genetic variants identified were interpreted by board-certified molecular geneticists and categorized as 'pathogenic,' 'presumed pathogenic,' 'variant of unknown significance,' 'presumed benign,' or 'benign,' according to the American College of Medical Genetics and Genomics (ACMG) standards. When assessing mutation frequencies of individual genes, 'pathogenic,' and 'presumed pathogenic' were counted as mutations while 'benign', 'presumed benign' variants and 'variants of unknown significance' were excluded.

Homologous recombination-related (HRR) genes determination

A combination of multiple test platforms was used to determine the mismatch repair deficiency (dMMRP) status of the tumors profiled, including MSI fragment analysis (FA, Promega, Madison, WI), IHC (MLH1, M1 antibody; MSH2, G2191129 antibody; MSH6, 44 anti-body; and PMS2, EPR3947 antibody [Ventana Medical Systems, Inc., Tucson, AZ, USA]) and NGS (for tumors tested with NextSeq platform, 7000 target microsatellite loci were examined and compared to the reference genome hg19 from the University of California). The three platforms generated highly concordant results as previously reported and in the rare cases of discordant results, the MSI or MMR status of the tumor was determined in the order of FA, IHC and NGS [5].

PD-L1 expression

Immunohistochemistry (IHC) was performed on full formalin-fixed paraffin-embedded (FFPE) sections of glass slides. Slides were stained using automated staining techniques, per the manufacturer's instructions, and were optimized and validated per CLIA/CAO and ISO requirements. The primary antibodies used against PD-L1 were 22c3 (pharmDx, Dako) and tumor proportion score (TPS) was calculated as the number of PD-L1 staining cells tumor cells divided by the total viable tumor cells, multiplied by 100. The tumor was considered positive if TPS ≥ 1% (high PD-L1 expression if TPS ≥ 50%).

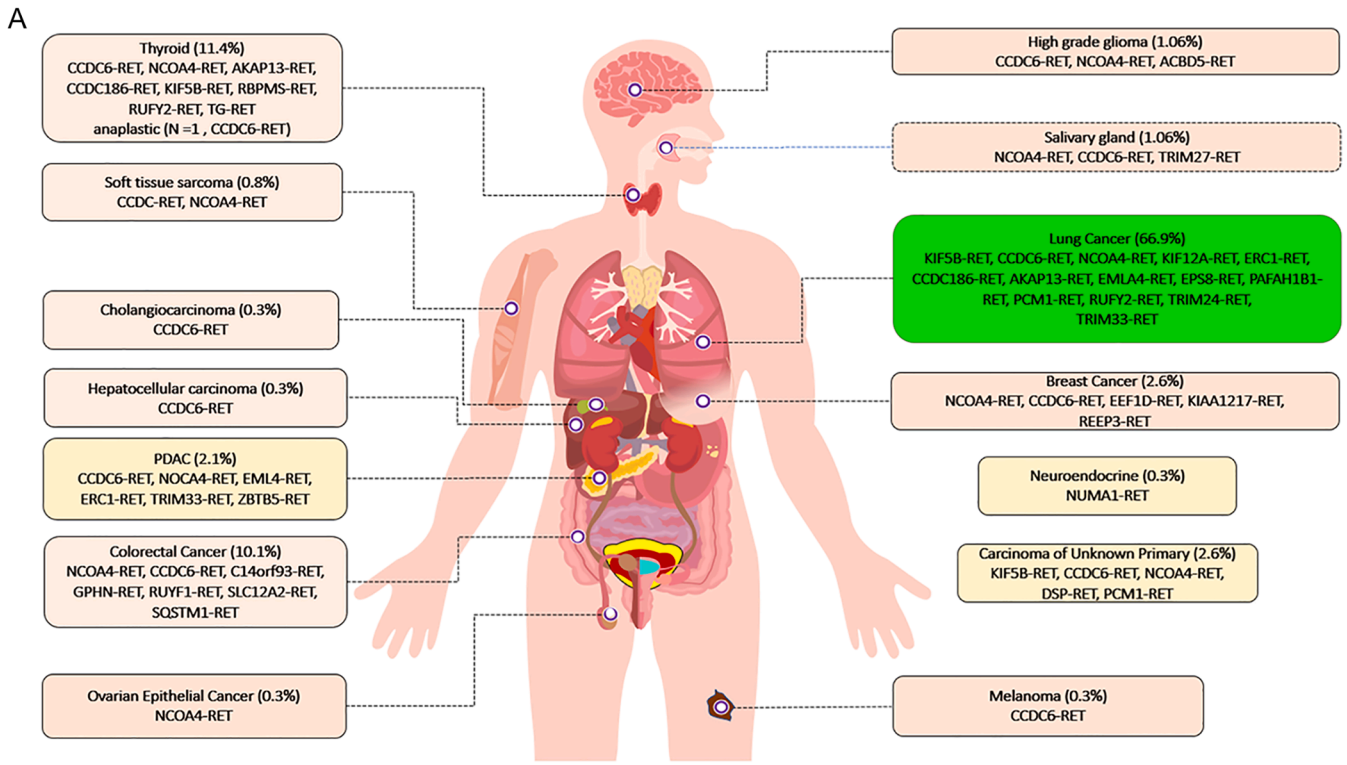
Tumor mutation burden (TMB)

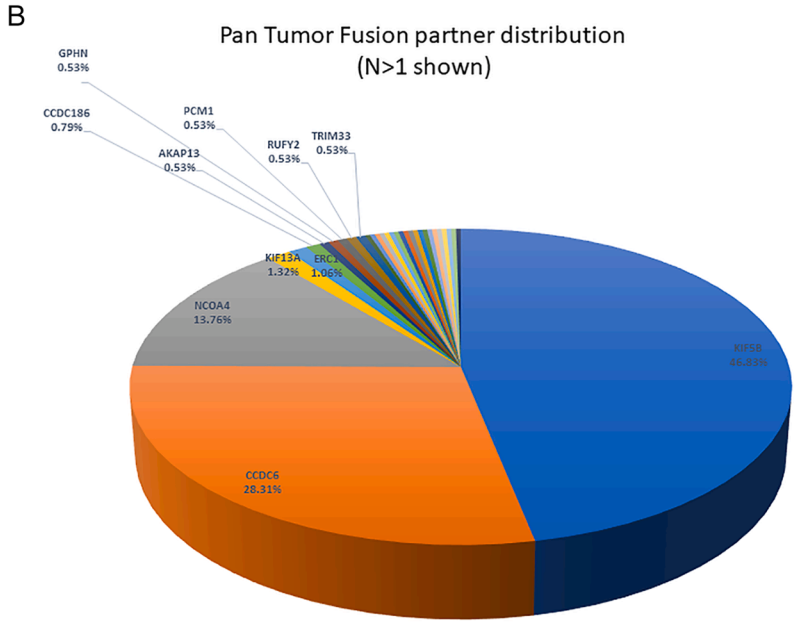
TMB was measured by counting all non-synonymous missense, nonsense, inframe insertion/deletion and frameshift mutations found per tumor that had not been previously described as germline alterations in dbSNP151, Genome Aggregation Database (gnomAD) databases or benign variants identified by Caris geneticists. A cutoff point of >=10 mutations per MB was used based on the KEYNOTE-158 pembrolizumab trial [6], which showed that patients with a TMB of ≥ 10 mt/MB across several tumor types had higher response rates (RR) than patients with a TMB of <10 mt/MB. Caris Life Sciences is a participant in the Friends of Cancer Research TMB Harmonization Project [7].

Results

Distribution and frequency of RET fusion positive (RET+) tumors

A total of 378 *RET*+ solid tumors were identified. The majority (84.7%, 320/378) were identified by WTS and the rest were identified by targeted NGS RNA sequencing (ARCHER). The most common *RET*+ sold tumors was NSCLC (66.9%, 253/378), followed by thyroid cancer (11.1%, 42/378), colorectal adenocarcinoma (10.1%, 38/378), breast cancer (2.6%, 10/378) and CUP (2.6%, 10/378) (Fig. 1A). The estimated frequency of *RET*+ samples within the specific tumor types was





RET Fusion partners	Archer	Transcriptome	Total	%
KIF5B	31	146	177	46.8%
CCDC6	20	87	107	28.3%
NCOA4	5	47	52	13.8%
KIF13A	1	4	5	1.3%
ERC1		4	4	1.1%
CCDC186		3	3	0.8%
AKAP13		2	2	0.5%
GPHN		2	2	0.5%
PCM1		2	2	0.5%
RUFY2		2	2	0.5%
TRIM33		2	2	0.5%
ACBD5		1	1	0.3%
C14orf93		1	1	0.3%
CGN		1	1	0.3%
DSP	1		1	0.3%
EEF1D		1	1	0.3%
EML4		1	1	0.3%
EMLA4		1	1	0.3%
EPS8		1	1	0.3%
KIAA1217		1	1	0.3%
NUMA1		1	1	0.3%
PAFAH1B1		1	1	0.3%
RBPMS		1	1	0.3%
REEP3		1	1	0.3%
RUFY1		1	1	0.3%
SLC12A2		1	1	0.3%
SQSTM1		1	1	0.3%
TG		1	1	0.3%
TRIM24		1	1	0.3%
TRIM27		1	1	0.3%
ZBTB5		1	1	0.3%
Total	58	320	378	

Fig. 1. A: Distribution of *RET* fusion positive tumors by tumor type (N = 378) B: Distribution of fusion partners of *RET*+ solid tumors C: Distribution of fusion partners among *RET*+ NSCLC D: Distribution of fusion partners among *RET*+ well-differentiated thyroid carcinoma, E: Distribution of fusion partners among *RET*+ colorectal adenocarcinoma F: Distribution of fusion partners among *RET*+ breast adenocarcinoma G: Distribution of fusion partners among *RET*+ CUP H: Distribution of fusion partners among *RET*+ pancreatic carcinoma.

about 0.7% (253 out of ~38,000) for NSCLC, 3.1% for thyroid cancer (42 out of ~1300), 0.2% for colorectal carcinoma (CRC) (38 out of ~23,000), and 0.1% for breast cancer (10 out of ~16,000). The clinical pathologic characteristics of the *RET*+ patients by tumor types are listed in Table 1.

Molecular characteristics of RET+ fusions

The most common fusion partners in all tumor types were *KIF5B* (46.8%) followed by *CCDC6* (28.3%) and *NCOA4* (13.8%) (Fig. 1B). The vast majority (97.4%, 368/378) of the fusion breakpoint occurred at exon 12 of the *RET*. The other fusion breakpoint occurred at exon 11

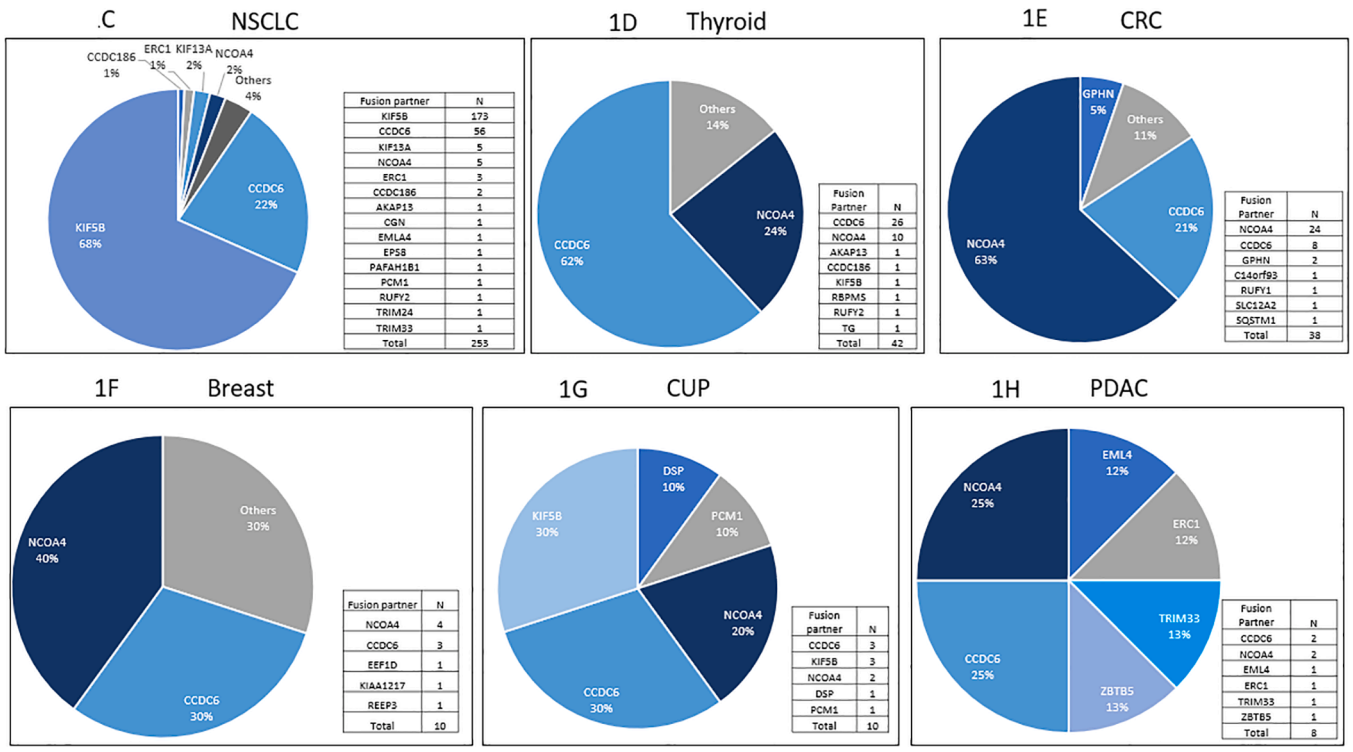


Fig. 1. (*continued*).

Table 1
Clinical pathologic characteristics of the *RET*+ patients by tumor types.

	NSCLC	Colorectal	Thyroid	Breast	CUP	Pancreatic
N	253	38	42	10	10	8
Age						
Median (range)	66 (27->89)	72.5 (34–88)	81.0 (9–84)	59.5 (35–75)	71.5 (41–87)	68.5 (55–81)
Mean (SD)	64.7 (12.02)	67.9 (12.7)	51.1 (21.1)	58.8 (15.7)	68.8 (13.9)	67.3 (9.0)
Sex (%)						
Male	113	15	14	0	6	5
Female	140	23	28	10	4	3
Fusion partner						
KIF5B-	173	0	1	0	0	0
CCDC6-	56	8	26	3	3	2
NCOA4-	5	24	10	4	2	2
ERC1-	3	0	0	0	0	1
KIF13A-	5	0	0	0	0	0
GPHN-	0	2	0	0	0	0
Sequencing methods						
Targeted RNA (Archer)	44	2	3	3	2	0
WTS	209	36	39	7	8	8
Mean junction read (SD)	45.2 (42.6)	22.7 (26.8)	18.7 (35.9)	16.7 (10.8)	48.8 (52.1)	38.4 (80.9)
PD-L1 expression (22C3)						
<1%	70	NA	NA	2	NA	NA
1–49%	71	NA	NA	2	NA	NA
>= 50%	92	NA	1	0	NA	NA
Unknown	20	38	41	6	10	8
TMB						
0–5	174	9	39	6	7	6
>5–10	55	4	0	4	2	2
>10	4	22	1	0	1	0
Unknown	20	3	2	0	0	0
Microsatellite						
Stable	244	14	41	10	9	8
Unstable	1	24	0	0	1	0

(1.9%, 7/378), exon 9 (0.5%, 2/378) and one fusion (0.3%) breakpoint at exon 10 of *RET* (Table 2).

Junctional read is the number of RNA reads that contained the fusion breakpoint. The mean junctional read per tumor sample was 53.5% +/- 10.4 standard deviation (SD). The mean junctional read per tumor sample was 45.2% +/- 42.6 standard deviation (SD) in NSCLC. The mean junctional read for thyroid cancer and colorectal cancer were 18.7% +/- 35.9 SD and 22.7% +/- 26.8, respectively.

The mean tumor mutation burden (TMB) for all *RET*+ tumors was 7.66 +/- 2.89 (SD). As *RET*+ CRC had an overall high TMB, the mean

Table 2

Fusionpartner	Fusionpartnerexon	RETexon	AnaplasticThyroidCarcinoma	BreastCarcinoma	CancerofUnknownPrimary	Cholangiocarcinoma	ColorectalAdenocarcinoma	HighGradeGlioma	LiverHepatocellularCarcinoma	LungNon – smallcelllungcancer(NSCLC)	Melanoma	Neuroendocrinetumors	OvarianSurfaceEpithelialCarcinomas	PancreaticAdenocarcinoma	SalivaryGlandTumors	SoftTissueTumors	ThyroidCarcinoma	Total
ACBD5	9	12						1										1
AKAP13	27	12								1							1	2
C14orf93	4	12					1											1
CCDC186	8	12								1								1
	16	12								1							1	2
CCDC6	1	12	1	3	2	1	5	1	1	51	1			2	1	2	25	96
	2	12			1		3	1		3								8
	8	12								1							1	2
	1 and intron 1	12								1								1
CGN	16	12								1								1
DSP	23	11			1													1
EEF1D	8	12		1														1
EML4	12	12												1				1
EMLA4	18	12								1								1
EPS8	12	12								1								1
ERC1	3	12								1								1
	15	12								1								1
	17	12								1				1				2
GPHN	8	12					2											2
KIAA1217	11	12		1														1
KIF13A	18	12								5								5
KIF5B	14	11								1								1
	15	11								1								1
		12			3					140							1	144
	16	12								15								15
	22	12								4								4
	23	12								8								8
	24	9								1								1
		11								1								1
		12								1								1
	16 and 15	12								1								1
NCOA4	6	12		1	1		1								2		1	6
	7	12		3			1			2						1	8	15
	8	11															1	1
		12					5						1					6
	9	12			1		17	1		3				2				24
NUMA1	22	9										1						1
PAFAH1B1	5	12								1								1
PCM1	28	11								1								1
		12			1													1
RBPMS	6	12															1	1
REEP3	5	12		1														1
RUFY1	14	12					1											1
RUFY2	9	12								1							1	2
SLC12A2	16	12					1											1
SQSTM1	3	10					1											1
TG	47	11															1	1
TRIM24	9	12								1								1
TRIM27	3	12													1			1
TRIM33	11	12								1				1				2
ZBTB5	2	12												1				1
Total			1	10	10	1	38	4	1	253	1	1	1	8	4	3	42	378

TMB in CRC not counting *RET*+ CRC was 7.48 +/- 13.80 (SD).

The most common single gene genomic alterations in *RET*+ solid tumors were *TP53* mutations at 34.8% (120/345) followed by *RASA1* (14.3%, 1/7) and *ARID1A* at 10.8% (27/250). The list of frequency of gene alterations are listed in Supplemental table 1 and Supplemental figure 1 and 2. The rest of the single gene genomic alterations occurred at a frequency < 15% (Supplemental table 2 and Supplemental figure 2). The relatively sparse spectrum for co-alterations may imply the lower genomic complexity of *RET*+ tumors and one could speculate that relationship to high responsiveness to *RET*+ tyrosine kinase inhibitors with the possible exception of *RET*+ CRC.

For global genomic alterations, loss of heterozygosity (LOH) occurred at 12.9% (9/70) followed by recombination-related genes (HRR) mutations at 11.9% (8/67) but number of samples tested was

fewer than most of the single gene alterations.

Gene amplification among *RET*+ solid tumors were rare with *MDM2* (3.7%), followed by *MYC* (2.5%), and *CDK4* (2.0%) (Supplemental table 3 and Supplemental figure 3).

RET+ *NSCLC*

RET+ NSCLC was the most common tumor types (66.9%). The most common *RET* fusion variant in NSCLC was *KIF5B-RET* at (68%) followed by *CCDC6-RET* at 22% (Fig. 1C). While not expressed in normal lung tissue, *KIF5B-RET* can be highly expressed in lung cancer tissue [8]. Based on a metanalysis including a total of over 8000 patients from 13 studies, the *KIF5B-RET* NSCLC genotype appear to have unique clinical features including higher frequencies in female and younger patients with no clear correlation to smoking history [9]. While there was no significant difference between sex, those with *RET* fusion NSCLC was significantly younger with a median age of 66 versus 69 in *RET* fusion positive versus negative NSCLC ($p<0.01$, data not shown). Of note, there were a total of 7 cases of concurrent *EGFR* mutatations (3 cases of L747_T751delinsP, 2 cases of E746_A750 del, 1 case each of V774N and E746_T751delinsA).

RET+ *thyroid cancer*

RET+ thyroid cancer was the second most common *RET*+ solid tumors identified in this study. The most common fusion partner was *CCDC6* in *RET*+ thyroid cancer (62%) and the only tumor type with *CCDC6* as the most common fusion partners (Fig. 1D). Of note, there were no concurrent BRAF mutations or NTRK fusions with *RET*+ thyroid cancers. Out of the 42 *RET*+ thyroid cancers, the majority was papillary ($n = 38$) followed by thyroid NOS ($n = 3$) and medullary ($n = 1$). There was one case of anaplastic thyroid cancer with the fusion partner of *CCDC6* which may have transformed from *RET*+ well differentiated thyroid cancer.

RET+ *colorectal cancer (CRC)*

RET+ colorectal cancer constituted the third most common tumor types among *RET*+ solid tumors. The most common RET fusion variant in CRC was *NCOA4-RET* at (63%) followed by *CCDC6-RET* at 21% (Fig. 1E). Of note, there were one case each of concurrent BRAF and KRAS mutations with *RET*+ CRC. In contrast to NSCLC where *RET*+ was associated with younger age, those with *RET* fusion positive CRC was significantly older with a median age of 72.5 versus 62 in *RET* fusion positive versus negative CRC ($p<0.01$, data not shown). While there was no significant difference between sex and RET status in CRC (data not shown). The median TMB of *RET*+ CRC on the other hand was high at 20.0 (Fig. 2A). Additionally, 63.2% (24/38) of the *RET*+ CRC was MSI-H. Twenty-one out of 22 TMB high (>10mt/base) *RET*+ CRC had MSI-H. Of the 7 *RET*+ CRC patients that underwent HRR testing, 4 were positive. All 4 of these HRR positive *RET*+ CRC patients had high TMB and were MSI-H. No apparent correlation between *RET* fusion partner and MSI status were observed with *NCOA4-RET* (62.5%, 15/24) in MSI-H versus (64.3, 9/14) MSI-stable (MSI-S). The mean junctional read of RET fusion was 22.7% +/- 26.8 SD, which was lower compared to *RET*+ NSCLC.

RET+ *breast cancer*

A total of 10 *RET*+ breast cancer samples were identified at a frequency of about 0.1%. The average age of the patients were 59.5 years old which was the youngest among the major tumor types identified harboring *RET* fusions (Table 1). The median TMB for these 10 breast cancer tumors was 5.0 (Fig. 2A). Out of these patients, 63% (5/8) were ER positive, 38% (38) were PR positive, while notably none had HER2 over-expression/amplification (0%, 0/8). Although with limited numbers, *NCOA4*- was the most common fusion partner but *CCDC6*-, *REEP3, EEF1D and KIAA1217* fusion partners were also identified (Fig. 1F).

RET+ *CUP*

A total of 10 *RET*+ CUP were identified. Median age was 71.5 (Table 1). The most common fusion partners were *KIF5B*- and *CCDC6*- (Fig. 1G). This may imply that in at least some of these cases of CUP, the primary site of tumor may have derived from NSCLC or thyroid, as *KIF5B*- was the most common fusion partner for NSCLC and *CCDC6*- was that of thyroid cancer.

RET+ *pancreatic cancer*

A total of 8 *RET*+ pancreatic adenocarcinoma was identified. These were all KRAS wild type pancreatic adenocarcinomas. The average age was 68.5 years old (Table 1). The median TMB was 4.5. The number of samples were limited and the most common fusion partner identified was *NCOA4*- and *CCDC6*- (Fig. 1H).

Other RET+ *solid tumors*

Four *RET*+ glioblastoma (two with *CCDC6*- and one each of *NCOA4*- and *ACBD5*-), four *RET*+ salivary gland cancer (two *NCOA4*- and one each of *CCDC6*-and *TRIM27*-), three *RET*+ soft tissue tumors (two *CCDC6*- and one *NCOA4*-) were identified. A single *RET* fusion variant was detected in an anaplastic thyroid carcinoma (*CCDC6-RET*), a cholangiocarcinoma (*CCDC6-RET*), one hepatocellular carcinoma (*CCDC6-RET*), melanoma (*CCDC6-RET*), neuroendocrine tumor (*NUMA1*) and in ovarian epithelial carcinoma (*NCOA4-RET*).

Discussion

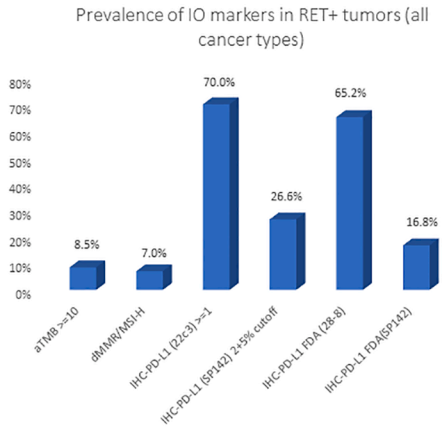
Our pan-tumor *RET* fusion survey identified *RET* fusions in 15 distinct tumor types including CUP. While 78% of the *RET* fusions were identified in NSCLC and well-differentiated thyroid cancers, the remaining *RET* fusion positive tumors consisted of colorectal adenocarcinoma, which was the third most common *RET*+ solid tumor in our survey, followed by breast adenocarcinoma, CUP, pancreatic adenocarcinoma, salivary gland carcinoma, glioblastoma, soft tissue tumors, hepatocellular carcinoma, cholangiocarcinoma, neuroendocrine tumor and ovarian tumors. Additionally, besides well-differentiated thyroid carcinoma, one case of anaplastic thyroid carcinoma was also identified.

Given the recent FDA approval to expand the use of selpercatinib to *RET*+ solid tumors outside of the more common NSCLC and thyroid cancers [10–13], it is of particular importance to raise awareness of RTK fusions in solid tumors.

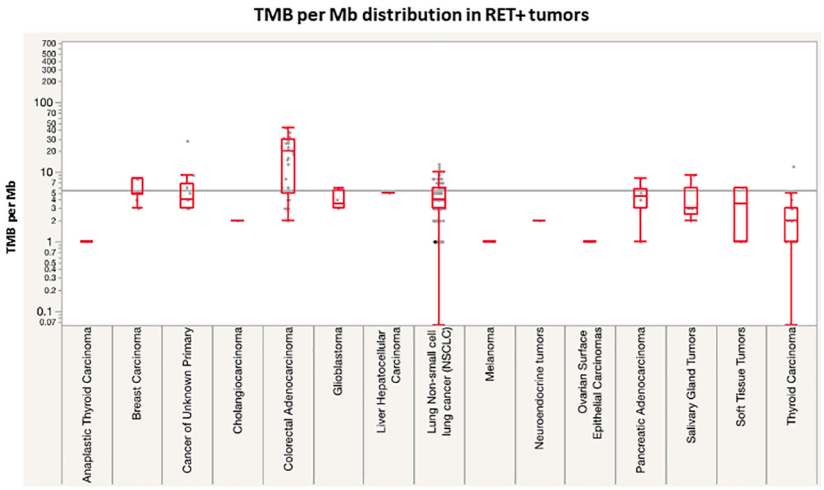
Although the most common fusion partner to *RET* was *KIF5B* accounting for close to half of the fusion partners of the *RET* fusion identified, there seemed to be a tumor specific dominance of one fusion partner: *KIF5B* for *RET*+ NSCLC, *CCDC6* for *RET*+ thyroid and *NCOA4* for *RET*+ colon and breast cancer. In the 10 *RET*+ CUP patients, there were 3 each of *KIF5B* and *CCDC6*. Thus, there could be a potential for using fusion partners as a hint to identify tumor of unknown origin, if there were to be ambiguity in the tumor tissue of origin.

Colorectal adenocarcinoma was the third most common *RET*+ solid tumor in our survey. Interestingly, Chan and colleagues demonstrated that the ectopic expression of a novel variant of the *NCOA4-RET* fusion gene promoted cell proliferation *in vitro* and *in vivo*, and the growth was suppressed by *RET* kinase inhibitors, showing that receptor tyrosine kinase fusions could act as a significant alternative driver in the development of colorectal cancer [14].

One of the important findings in this report was that *RET*+ CRC has a very high tumor mutation burden and a high proportion with MSI-High (Fig. 2A). This observation extends the finding of *NTRK*+ CRC also



Test	Positive	Negative	Total	%
aTMB >=10	30	323	353	8.5%
dMMR/MSI-H	26	343	369	7.0%
IHC-PD-L1 (22c3) >=1	166	71	237	70.0%
IHC-PD-L1 (SP142) 2+5% cutoff	29	80	109	26.6%
IHC-PD-L1 FDA (28-8)	75	40	115	65.2%
IHC-PD-L1 FDA(SP142)	19	94	113	16.8%



Cancer Type	Median TMB
Anaplastic Thyroid Carcinoma	1.0
Breast Carcinoma	5.0
Cancer of Unknown Primary	4.0
Cholangiocarcinoma	2.0
Colorectal Adenocarcinoma	20.0
Glioblastoma	3.5
Liver Hepatocellular Carcinoma	5.0
Lung Non-small cell lung cancer (NSCLC)	4.0
Melanoma	1.0
Neuroendocrine tumors	2.0
Ovarian Surface Epithelial Carcinomas	1.0
Pancreatic Adenocarcinoma	4.5
Salivary Gland Tumors	3.0
Soft Tissue Tumors	3.5
Thyroid Carcinoma	2.0

Fig. 2. A: Box and whiskers plot of TMB among the major *RET*+ solid tumors.

having a high TMB [15]. Similarly, Yakirevich and colleagues reported 9 cases of colorectal cancer harboring *ALK* gene fusions which predominantly involved the proximal colon and often exhibited MSI [16]. While we await further evaluation on the association of kinase fusions with microsatellite instability in colorectal cancers, this finding cautions us to confirm the MSI status when a fusion is detected or vice versa. This issue may also have treatment implications. The efficacy of selpercatinib in tumors other than lung or thyroid was reported recently by Subbiah and colleagues and demonstrated the low RR of *RET*+ CRC which was at 20.0% ($n = 10$) when compared to their total cohort of RR 43.9% ($n = 41$) [17].

This could be explained at least in part by the tumor heterogeneity of CRC and the fact that as shown in our study, *RET*+ CRC tend to be associated with MSI high status, which is known for poor prognosis [18]. Although the small number of patients makes the assessment challenging, one must be cautious on the "blanket" use of *RET* TKIs in this setting of *RET*+ CRC, where perhaps immunotherapy and other options should also be considered.

In our survey, breast cancer followed colon cancer with 10 cases of *RET* fusions. In a large survey of *RET* alterations among 9693 breast adenocarcinoma that utilized hybrid-capture DNA sequencing, 8 *RET* fusions were identified (*CCDC6-RET* [$N = 6$], *NCOA4-RET* [$N = 1$], *RASGEF1A-RET* [$N = 1$] [19]). It appears that the majority of the *RET*+ breast cancer were ER negative and negative for *HER2* amplification which was consistent with our survey. *RET* fusions in breast adenocarcinoma are thought to be oncogenic and indeed, one patient had a rapid clinical and radiographic response to cabozantinib, a multi-target *RET* kinase inhibitor [19].

While there were only 8 cases of *RET* positive pancreatic cancer in our survey, identification of potentially targetable alterations would be of significant value given the grave prognosis of pancreatic cancer. Utilizing FISH, Chou and colleagues have reported the identification of *RET* gene rearrangements in 3 out of 36 (8.3%) pure pancreatic acinar cell in which one of them was *CCDC6-RET* [20]. In our survey, while most of the histology reported were "pancreatic ductal adenocarcinoma", it is possible that there may have been a classification error at the time of test requisition form completion and some of the patients may have had "pancreatic acinar cell carcinoma", which appears to have a different biology when compared to the traditional ductal adenocarcinomas [21].

The prevalence of *RET* fusions were relatively low in this large cohort. One of the limitation of this study is the fact that there may be selection bias in those who were offered molecular testing. For example, it is possible that patients who were locally tested positive for KRAS mutant colorectal cancer or BRAF mutant thyroid cancer were not offered broad molecular testing and this may have skewed the overall incidence of *RET* fusions in certain tumor types in our study. Another limitation of this study is the lack of detailed clinical information regarding the timing of when the molecular analysis was performed (i.e. stage, pre vs post treatment evaluation). Outcomes were inferred based on time from tissue collection to date of last contact. In reality, NGS was performed at varying time points during the course of the disease and treatments. Although we were unable to distinguish if *RET* fusions were baseline characteristics prior to any treatment or if it actually reflected acquired resistance, there were a total of 7 cases of concurrent EGFR mutatations (3 cases of L747_T751delinsP, 2 cases of E746_A750 del, 1 case each of V774N and E746_T751delinsA), impling the possibility of *RET* fusions as a resistance mechanism.

Despite these limitations, we were able to determine the characteristics of *RET* fusions in a tumor agnostic manner. While Zhou and colleagues have also published on pan-tumor *RET* alterations using the cbioportal genomic database, they captured *RET* alterations including mutations and amplifications in addition to the fusions [22]. Finally, a more detailed examination of the clinical effects of other co-existing mutations along with underlying biological and molecular mechanism to account for the differences in survival outcomes of various *RET* fusions is eagerly awaited.

In conclusion, we believe that highly actionable alteration notable in multiple tumor types continues to highlight the need for broad panel testing for advanced cancers including RNA NGS and WES and tissue agnostic treatment approaches.

Executive summary of pan tumor *RET* fusion survey

(1) 15 different tumor types and CUP with in-frame *RET* fusions as detected by NGS RNA sequencing
(2) 31 different fusion partners in *RET* fusion solid tumors
(3) A total of 378 *RET*+ solid tumors were identified. The majority (84.7%, 320/378) were identified by WTS and the rest were identified by targeted NGS RNA sequencing (ARCHER).
(4) The majority of *RET* fusions were seen in the following tumor types: NSCLC (66.9%), thyroid cancer (11.1%), colorectal (10.1%)
(5) The estimated frequency of *RET* fusions within specific tumor types were NSCLC 0.7%, thyroid cancer 3.1%, colorectal cancer 0.2% and breast cancer 0.1%
(6) Three major fusion partners: *KIF5B* (46.8%), *CCDC6* (28.3%), *NCOA4* (13.8%). Although *KIF5B* is the most common fusion partner, it is mostly found in NSCLC while *CCDC6* and *NCOA4* are identified in almost all *RET* fusion positive (*RET*+) solid tumors
(7) Different dominant fusion variants among major tumor types.
  (a) *KIF5B-RET* (NSCLC)
  (b) *NCOA4-RET* (colorectal, breast)
  (c) *CCDC6-RET* (Thyroid)
(8) High PD-L1 expression ≥50% (22C3) accounted for 36.4% of *RET*+ NSCLC
(9) *RET*+ CRC had a high median TMB and were commonly MSI-H.

CRediT authorship contribution statement

Misako Nagasaka: Conceptualization, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. **Danielle Brazel:** Formal analysis, Investigation, Writing – review & editing. **Yasmine Baca:** Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – review & editing. **Joanne Xiu:** Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – review & editing. **Mohammed Najeeb Al-Hallak:** Formal analysis, Investigation, Writing – review & editing. **Chul Kim:** Formal analysis, Investigation, Writing – review & editing. **Jorge Nieva:** Formal analysis, Investigation, Writing – review & editing. **Jeffrey J. Swensen:** Formal analysis, Investigation, Writing – review & editing. **David Spetzler:** Formal analysis, Investigation, Writing – review & editing. **Wolfgang Michael Korn:** Formal analysis, Investigation, Writing – review & editing. **Mark A. Socinski:** Formal analysis, Investigation, Writing – review & editing. **Luis E. Raez:** Formal analysis, Investigation, Writing – review & editing. **Balazs Halmos:** Formal analysis, Investigation, Writing – review & editing. **Sai-Hong Ignatius Ou:** Conceptualization, Formal analysis, Investigation, Methodology, Project administration, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.tranon.2023.101744.

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