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LETTER TO THE EDITOR

JAK Pseudokinase Domain Variants Highlight nRTK VUSs Identified with Next-Generation Sequencing in Solid Tumor Patients

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Main Text

Non-receptor tyrosine kinase (nRTK) pathways are aberrantly activated in cancer, and mutations in nRTKs have potential therapeutic and prognostic importance. Consisting of 10 families, the 32 known human nRTKs each include a TKD made of N and C-terminal lobes necessary for catalytic activity, as well as varying regulatory regions including Src homology 2 (SH2) and 3 (SH3), and in the case of the Janus kinases a PSKD which is also bi-lobed [1]. Tumor profiling with NGS enables the entire coding sequence of numerous genes to be evaluated, thus facilitating the identification of novel nsSNPs in nRTKs.

We reviewed advanced breast, colon and lung cancer patients treated at West Cancer Center (Memphis, Tennessee) from 2013 to 2015 who received tumor profiling including NGS with a 592 cancer-related gene panel from Caris Life Sciences (Phoenix, Arizona). Caris NGS searched 14 nRTKs: *ABL1*, *ABL2*, *AKT1*, *AKT2*, *AKT3*, *BTK*, *JAK1*, *JAK2*, *JAK3*, *SRC*, *CDK4*, *CDK6*, *CDK12*, *PIK3CA*. All mutations test-defined as either pathogenic (PATH) or non-synonymous single nucleotide variants deemed variants of undetermined significance (VUS) were included. All variants had >99% detection confidence based upon allele frequency and amplicon coverage. In order to classify VUS, *in silico* analysis with PolyPhen-2 was utilized to predict pathogenicity [2]. Any VUS predicted-damaging with *in silico* analysis we denote VUSp. VUSs were then classified as occurring within or outside of the TKD; PSKD lesions were also detailed for *JAK1–3*.

346 patients (79 breast, 110 colon and 157 non-small cell lung cancer (NSCLC)) were identified. The cohort had a median age of 61 years (range 26–86). 58% were female; 62% were Caucasian and 35% African-American. 245 variants were found, with 200 VUS and 45 PATHs. PATHs were seen in 2 genes: *PIK3CA* (21 breast, 13 colon, 5 NSCLC) and *AKT1* (6 breast). 168/346 (49%) patients had ≥1 nRTK lesion. 52/200 (26%) VUS were VUSp and spread amongst 48 patients (5 breast, 13 colon and 30 NSCLC). VUSp were found in 13/14 nRTKs (excluding *AKT1*) with median 3 (range 0–10). The most numerous VUSp by gene were *JAK3* (10), *ABL1* (8), *JAK2* (5), *BTK* (5) and *CDK12* (5). By cancer type, the most-frequently mutated nRTKs were: *SRC* (2/2 VUS were VUSp) and *ABL2* (1/5) in breast, *ABL1* (5/10), *JAK3* (3/27) and *CDK12* (2/8) in colon, and *JAK3* (6/20), *BTK* (5/8), *ABL1* (3/12) and *JAK2* (3/11) in NSCLC. Of 180 VUS with *in silico* results, 68% were outside of the TKD (29/122 VUSp), 23% TKD-restricted (13/42) and 9% in PSKD of *JAK1–3* (11/16).

Of note, 44 unique VUS were found in *JAK1–3*, with a total 18 VUSp (3 *JAK1*, 5 *JAK2* and 10 *JAK3*). 12/18 *JAK* VUSp were NSCLC, including 9 PSKD, 2 FERM (4.1, Ezrin, Radixin, Moesin) and 1 TKD variants (Table 1). Comprised of 4 regions including N-terminal FERM and SH2 domains and C-terminal PSKD and TKD, the JAK family is known to harbor oncogenic mutations in myeloproliferative neoplasms (e.g. V617F in JAK2), other hematologic malignancies and several solid cancers [1]. While described in all 4 domains, the majority of activating JAK mutations focus in the N-lobes of the PSKD and TKD, which normally form an autoinhibitory

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Table 1 *JAK1–3* VUS in NSCLC patients predicted-damaging with *in silico* analysis

JAK	VUS; allele frequency (Caris)	Location	Accession Number; Minor allele frequency (ExAC)	Histology	Age, race, gender	Genomics (EGFR, KRAS, ALK or ROS1-rearranged, PDL1 (%))
JAK1	D660N; 66%	PSKD; N-lobe	rs368904859; *T* = 2.0e-5	Adeno-carcinoma	66, C, M	Negative
	P674S; 9%	PSKD; N-lobe	None	Squamous	76, C, M	PDL1+ (5%)
	D739N; 47%	PSKD; N-lobe	rs759709239; *T* = 3.3e-5	Large cell	43, C, M	KRAS+
JAK2	E621D; 30%	PSKD; N-lobe	None	Unspecified	65, AA, M	Negative
	D686H; 13%	PSKD; N-lobe	None	Adeno-carcinoma	55, C, M	Negative
	C1105F; 41%	TKD; C-lobe	None	Adeno-carcinoma	73, C, F	KRAS+, ROS1-rearranged
JAK3	V55E; 13%	FERM	None	Adeno-carcinoma	74, C, F	Negative
	Y105H; 21%	FERM	None	Squamous	68, C, F	PDL1+ (20%)
	R537Q; 47%	PSKD; N-lobe	rs587778413; *T* = 4.1e-5	Adeno-carcinoma	60, C, F	PDL1+ (65%)
	L702P; 53%	PSKD; C-lobe	rs772117537; G = 1.7e-5	Squamous	80, C, M	Negative
	P745L; 50%	PSKD; C-lobe	rs776106625; A = 8.3e-6	Adeno-carcinoma	68, C, M	EGFR+ (E746_A750del)
	L788I; 7%	PSKD; C-lobe	None	Squamous	68, AA, M	Negative

interface [1]. Further models have also detailed a second interaction between the C-lobes of the PSKD and TKD [3]. Our cohort contained 6 N-lobe PSKD, 3 C-lobe PSKD and 1 C-lobe TKD pnsSNPs which may impact domain interactions leading to *JAK* activation and need further examination in NSCLC. 6/12 of these *JAK* mutations were in NSCLC patients whose tumors were EGFR-/KRAS-/ALK-/ROS-/PDL1-. A search of public databases revealed only 5/12 NSCLC *JAK* VUS were reported on dbSNP and ExAC, and only 1 was found in COSMIC (*JAK1* D660N, ID: COSM4693744). Additionally, *JAK3* P745L has been previously reported as a somatic PSKD mutation in cutaneous T-cell lymphoma (CTLA), where the authors demonstrated that ruxolitinib inhibited DNA synthesis and induced apoptosis in *JAK1* and *JAK3* PSKD-mutated CTLA cells [4].

Overall, we utilized NGS as a tool to discover nRTK VUS in solid tumor patients that warrant further evaluation. In the cohort, 13% of breast, colon and NSCLC patients had a VUSp with 39/52 (75%) occurring outside of the TKD-proper, signifying the need to localize these variants to their respective regulatory domains in order to predict potential functional impact. The majority of *JAK1–3* VUSp localized to the PSKD in NSCLC patients; the frequency of solid tumor *JAK* PSKD mutations should be examined on a larger scale as they could be amenable to currently approved therapies or investigational targets.

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