






## ARTICLE



# Activating *IGF1R* hotspot non-frameshift insertions define a novel, potentially targetable molecular subtype of adenoid cystic carcinoma

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Activation of the tyrosine kinase receptor IGF1R is targetable with existing tyrosine kinase inhibitors (TKIs) and monoclonal antibodies, but mutations in *IGF1R* have not been systematically characterized. Pan-cancer analysis of 326,911 tumors identified two distinct, activating non-frameshift insertion hotspots in *IGF1R*, which were significantly enriched in adenoid cystic carcinomas (ACCs). *IGF1R* alterations from 326,911 subjects were analyzed by variant effect prediction class, position within the gene, and cancer type. 6502 (2.0%) samples harbored one or more alterations in *IGF1R*. Two regions were enriched for non-frameshift insertions: codons 663–666 at the hinge region of the fibronectin type 3 domain and codons 1034–1049 in the tyrosine kinase domain. Hotspot insertions were highly enriched in ACCs (27.3-fold higher than in the remainder of the pan-cancer dataset;  $P = 2.3 \times 10^{-17}$ ). Among salivary gland tumors, *IGF1R* hotspot insertions were entirely specific to ACCs. *IGF1R* alterations were most often mutually exclusive with other ACC drivers (9/15, 60%). Tumors with non-frameshift hotspot *IGF1R* insertions represent a novel, potentially targetable subtype of ACC. Additional studies are needed to determine whether these patients respond to existing IGF1R inhibitors.

*Modern Pathology*; <https://doi.org/10.1038/s41379-022-01126-3>

## INTRODUCTION

Adenoid cystic carcinoma (ACC) is among the most prevalent malignant salivary gland tumors and typically follows a slow but progressive clinical course with poor long-term prognosis<sup>1</sup>. Tumors are biphasic with a basaloid appearance and classically demonstrate cribriform architecture with punched out spaces containing hyaline material, although tubular and solid growth patterns may be present<sup>2–4</sup>. There is significant histological overlap between ACC and other basaloid salivary gland neoplasms, yielding challenging diagnostic dilemmas, particularly on small biopsies and fine needle aspirations<sup>5</sup>.

Recurrent genomic alterations of primary and metastatic ACC have been well studied<sup>6</sup>. The majority harbor driver *MYB* or *MYBL1* fusions, with *NFIB* as the most frequent partner gene<sup>7,8</sup>. Activating alterations in the *NOTCH* gene family including genes that activate the Notch-signaling pathway (inclusive of *SPEN* and *FBXW7*), are common and associated with a worse prognosis<sup>9</sup>. Furthermore, a subset of cases harbor potentially targetable kinase alterations<sup>6,10</sup>. A small proportion of cases do not harbor any of these classic alterations, raising the possibility of an alternative, yet to be characterized driver.

Insulin-like growth factor 1 receptor (*IGF1R*) is a receptor tyrosine kinase that signals through the PI3K-AKT and Ras-MAPK pathways to promote cell survival and proliferation. *IGF1R* has recently been

shown to play a significant role in ACC biology via signaling through an autocrine loop involving the classic *MYB-NFIB* fusion<sup>11,12</sup>. While *IGF1R* alterations have been reported at low frequencies across a range of tumor types<sup>13,14</sup>, no systematic pan-cancer genomic studies have demonstrated a clear contribution to tumorigenesis. Furthermore, few specific pathogenic *IGF1R* variants have been characterized. Existing IGF1R inhibitors have failed to demonstrate efficacy in cancers with elevated signaling in the IGF1R axis, but they have never been tested in patients with activating mutations of *IGF1R* itself. In this study, we describe a novel subset of ACC with potentially targetable *IGF1R* hotspot mutations.

## METHODS

### Design, setting, and participants

We performed a retrospective cohort study (outline of design in Supplementary Fig. S1) whose solid tumor specimens were submitted for comprehensive genomic profiling (CGP) in the course of clinical care between 2012-09-27 and 2021-09-29 at Foundation Medicine, Inc. in Cambridge, MA and Morrisville, NC. The study was approved by the Western Institutional Review Board (Protocol No. 20152817) and reported in accordance with the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guidelines. All patients with *IGF1R* single nucleotide variants, indels, rearrangements, or copy number alterations (amplifications and biallelic deletions) were included in

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Received: 30 March 2022 Revised: 1 June 2022 Accepted: 3 June 2022

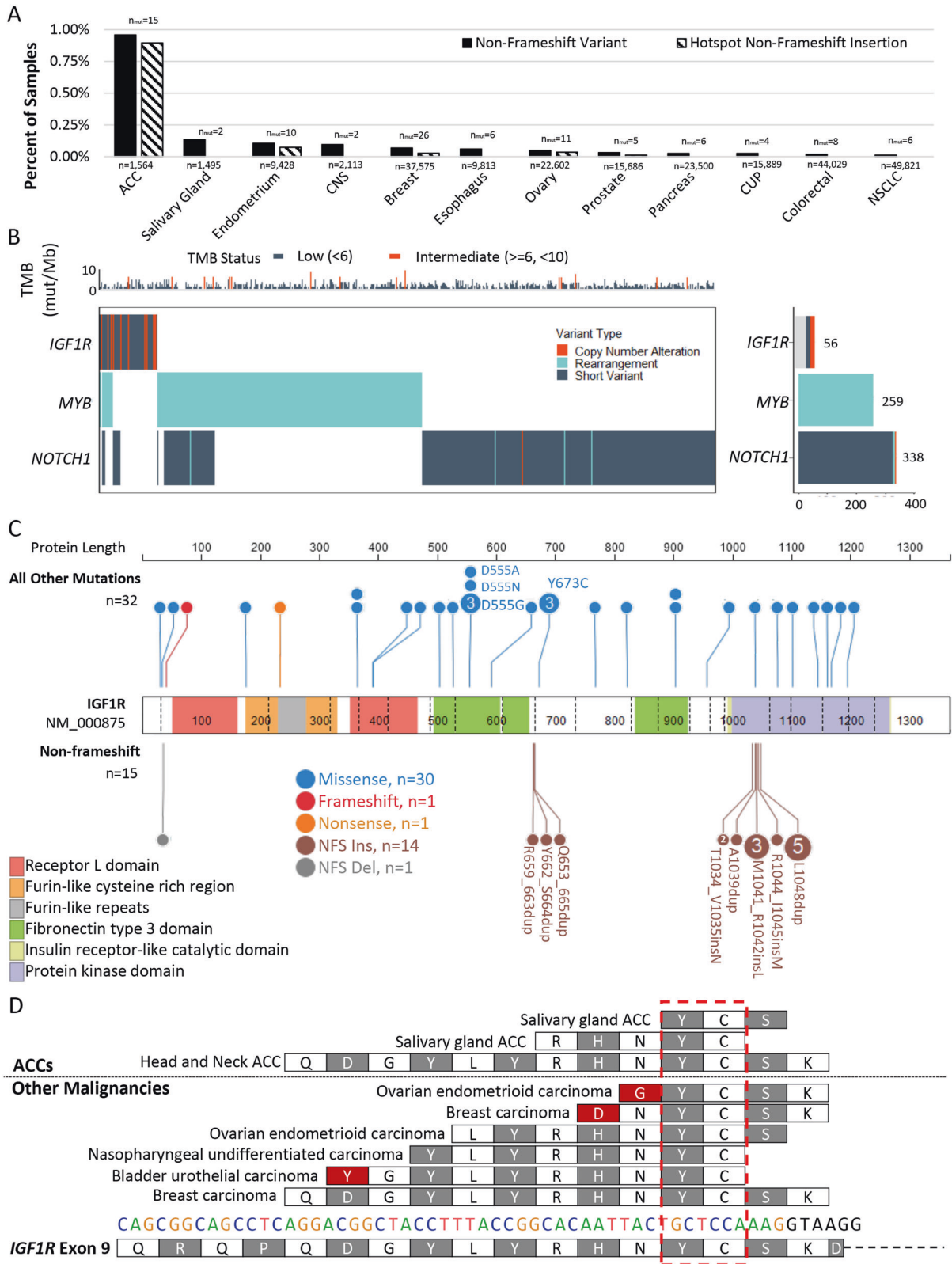
Published online: 15 August 2022

downstream analyses. All patients without *IGF1R* variants from the same period were used for comparisons.

**DNA sequencing and analysis**

To detect *IGF1R* genomic alterations, CGP was performed on formalin-fixed, paraffin-embedded tissue in a Clinical Laboratory Improvement

Amendments (CLIA)-certified, College of American Pathologists (CAP)-accredited, New York State-approved laboratory, as previously described<sup>15</sup>. Sections were macrodissected to achieve >20% estimated percent tumor nuclei (%TN) in each case, where %TN = number of tumor cells divided by total number of nucleated cells. In brief, DNA was extracted, quantified, and enriched via adaptor ligation hybrid capture for all coding exons of up



**Fig. 1 ACC-oriented landscape of IGF1R non-frameshift alterations.** **A** Frequency of IGF1R non-frameshift mutations by cancer type. Hotspot alterations were 12.1-fold more frequent in ACC than any other tumor type and 27.3-fold more frequent when compared to all non-ACC cases in the pan-cancer dataset ( $P = 2.3 \times 10^{-17}$ ). Cases with high TMB ( $>10$  mut/Mb) were excluded from the analysis. Only cancer types with  $\geq 1000$  samples and at least two total IGF1R non-frameshift alterations are shown. **B** Co-occurrence patterns for ACCs, stratified by driver alteration status (*MYB* fusion, *NOTCH1*, *IGF1R*). Of 1564 ACC samples, 55 harbored IGF1R alterations, of which 37 were mutually exclusive with *MYB* or *NOTCH1* drivers. **C** Spectrum of IGF1R somatic single nucleotide and indel mutations in ACC. Non-frameshift indels were concentrated in hotspots at amino acids 663–666 and 1034–1049, corresponding to the fibronectin type 3 domain and tyrosine kinase domain, respectively. **D** Configuration of non-frameshift duplication events in the exon 9 hinge region of the fibronectin type 3 domain for ACC (upper) and non-ACC (lower) samples. Between three and 13 amino acids were duplicated and all included Y262 and C263 (red dashed box). ACC Adenoid cystic carcinoma, CNS Central nervous system, CUP Carcinoma of unknown primary, NSCLC Non-small cell lung carcinoma.

**Table 1.** Clinical and genomic characteristics of ACCs with IGF1R hotspot non-frameshift insertions.

Tissue of origin	Sex	Age	Clinical stage	IGF1R non-frameshift insertion	IGF1R functional domain	VAF	Tumor purity
Salivary gland	F	60	IV	R659_C663dup	Fibronectin 3 hinge	36.0%	40.0%
Salivary gland	F	70	IV	Y662_S664dup	Fibronectin 3 hinge	33.5%	58.3%
Head and neck	M	44	IV	Q653_K665dup	Fibronectin 3 hinge	17.5%	28.3%
Salivary gland	F	66	N/A	T1034_V1035insN	Kinase	39.0%	68.5%
Head and neck	M	50	N/A	A1039dup	Kinase	45.0%	50.0%
Head and neck	M	51	N/A	M1041_R1042insL	Kinase	12.3%	31.5%
Lung	F	26	IV	M1041_R1042insL	Kinase	38.8%	70.1%
Breast	F	68	IV	M1041_R1042insL	Kinase	25.6%	31.8%
Head and neck	F	59	IV	R1044_I1045insM	Kinase	7.1%	30.1%
Salivary gland	F	61	IV	L1048dup	Kinase	48.0%	69.5%
Head and neck	M	62	N/A	L1048dup	Kinase	31.8%	61.1%
Salivary gland	M	63	IV	L1048dup	Kinase	19.3%	18.1%
Salivary gland	F	51	N/A	L1048dup	Kinase	31.3%	59.7%
Head and neck	F	61	IV	L1048dup	Kinase	34.9%	50.7%

IGF1R transcript = NM\_000875.

N/A not available, VAF variant allele fraction.

to 324 cancer-related genes and select introns from up to 34 genes frequently rearranged in cancer. Sequencing of captured libraries was performed using the Illumina sequencing system to a mean exon coverage depth of targeted regions of  $>500\times$ . Processed and aligned reads were analyzed for single nucleotide variants, indels, rearrangements, and copy number alterations. Tumor mutational burden (TMB, mutations/Mb) was determined on 0.8–1.1 Mb of sequenced DNA. TMB high ( $>10$  mut/Mb) samples were excluded from further analysis to avoid over interpretation of passenger mutations. Tumor purity was estimated based on genome-wide aneuploidy and allelic imbalance and averaged with pathology assessment of percent tumor nuclei. Pathology reports were reviewed to extract data on age, sex, and oncologic diagnosis. The pathologic diagnosis of each case was confirmed on routine hematoxylin and eosin (H&E)-stained slides according to the diagnostic criteria specified in the 2017 World Health Organization Classification of Head and Neck Tumors.

### Statistical analyses

Differences among categorical variables were assessed using Fisher's exact test. Statistical analyses were performed using R version 3.6.2 (R Foundation for Statistical Computing). Statistical tests were 2-sided and used a significance threshold of  $p < 0.05$ . Reported  $p$  values were adjusted for multiple testing using the False Discovery Rate (FDR) method.

## RESULTS

### Pan-cancer landscape of IGF1R mutations highlights hotspot non-frameshift insertions

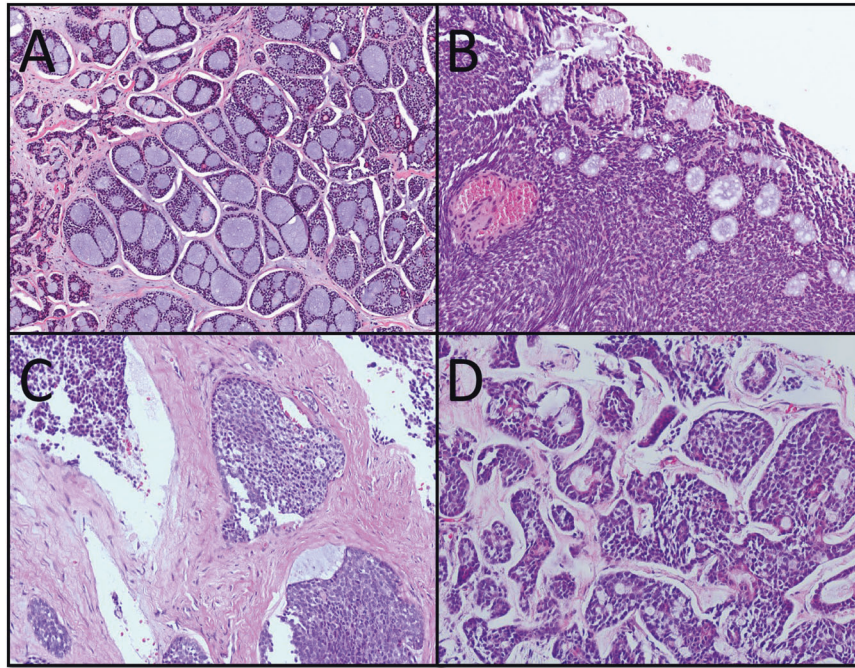
Tissue samples from 326,911 patients underwent CGP (Supplementary Fig. S1). Overall, 2.0% (6502/326,911) of samples exhibited at least one IGF1R alteration (Supplementary Fig. S2A, Supplementary Table S1). As observed in prior cancer landscape surveys, IGF1R alterations were seen in many cancer types and were most frequent in breast (4.0%, 1489/37,575), adenoid cystic

carcinoma (3.5%, 55/1564), and esophagus (3.3%, 324/9813) cancers (Supplementary Fig. S2B). The most common IGF1R mutations included missense mutations (52.4%, 3614/6899), amplifications (34.3%, 2366/6899), and rearrangements (6.3%, 436/6899) (Supplementary Table S1).

Review of the spatial distribution of pan-cancer IGF1R mutations revealed that single nucleotide alterations were dispersed throughout the gene. In contrast, rare non-frameshift insertions were localized to specific hotspots in the gene, involving either the kinase domain or the structurally critical hinge region of the fibronectin 3 domain (Supplementary Fig. S2A, lower track; Supplementary Table S2). Similar alterations are a well-described mechanism of activation for other kinases, but this phenomenon has not been previously described in IGF1R. Given the biological and therapeutic implications of activation of a kinase with a vaguely defined role in oncogenesis, these hotspot non-frameshift alterations were examined more closely.

### IGF1R hotspot insertions are enriched in adenoid cystic carcinomas and are less common in the setting of other driver mutations

IGF1R non-frameshift insertions were markedly enriched in ACC compared to other cancer types. Non-frameshift insertions were found in 0.96% (15/1564) of ACCs, a frequency that was 12.1-fold higher than any other tumor type with at least 1000 samples, and 27.3-fold higher than in the remainder of the pan-cancer dataset ( $P = 2.3 \times 10^{-17}$ ) (Fig. 1A). Of these, 14/15 (93.3%) occurred in two hotspots newly identified in this study. Characteristics of ACCs with hotspot non-frameshift insertions are found in Table 1. The majority (9/15, 60.0%) of ACC with IGF1R hotspot alterations did not co-occur with more common ACC driver alterations in MYB



**Fig. 2 Representative histology of salivary neoplasms reclassified as adenoid cystic carcinoma after identification of *IGF1R* hotspot non-frameshift insertions.** Tumors demonstrated basaloid cells with sharply demarcated matrix production, morphology compatible with adenoid cystic carcinoma. Cases included those with both *IGF1R* hotspot single nucleotide variants (panels A and C) and in-frame indel alterations (panels B and D).

and *NOTCH1* (Fig. 1B). Samples with non-frameshift hotspot insertions had co-occurring alterations involving *MYB* fusions (20.0%, 3/15), *NOTCH1* (26.7%, 4/15), and *HRAS* (13.3%, 2/15). *IGF1R* hotspot insertions occurring at the c-terminal end of exon 9 after the fibronectin 3 domain (codons 653–666) were found to be independent of other drivers (100%, 3/3), whereas kinase domain alterations were found both independently (50%, 6/12) as well as co-occurring with other drivers (50%, 6/12). These *IGF1R* hotspot insertions represent a previously unrecognized molecular subtype of ACC.

As in the pan-cancer samples, ACC non-frameshift *IGF1R* insertions were clustered in two distinct hotspots around amino acid (AA) positions 663–666 and 1034–1049 (Fig. 1C), corresponding to the fibronectin type 3 domain and tyrosine kinase domain, respectively. Additionally, recurrent missense alterations occurred at a hotspot at position D555 within the fibronectin type 3 domain.

The hinge region is a key structural component of the protein. A total of nine samples had non-frameshift insertions at this hotspot, including three ACCs. Insertions occurred in a very narrow three amino acid window in *IGF1R* exon nine and ranged from three to 13 AAs in length (Table 1 and Supplementary Table S1). All were duplications or near duplications that differed from reference only by substitution of a single amino acid at the duplication breakpoint (Fig. 1D). All cases shared duplication of Y662 and C663 with one to nine additional amino acids duplicated on either side, suggesting that insertion of these two amino acids may represent the minimum change required to alter protein function.

Non-frameshift insertions were also clustered at the N-terminal end of the kinase domain, with insertion start sites spanning amino acids 1034–1049 (Table 1 and Supplementary Table S1). Variants at this hotspot were more frequent, with 12 ACCs and 25 non-ACC samples exhibiting non-frameshift insertions in this region. Kinase non-frameshifts were a mix of duplications and non-duplication insertions, and in all cases resulted in net addition of one amino acid. The most common individual variant was L1048dup (five ACCs and 13 non-ACCs), followed by M1041\_R1042insL (three ACCs and two non-ACCs), and R1044\_I1045insM (one ACC and 3 non-ACCs).

### ***IGF1R* hotspot alterations in salivary gland neoplasms suggest an ACC diagnosis**

After identification of an association between ACC and hotspot alterations, histology was retrospectively re-reviewed for all tumors of salivary, head and neck, breast, and lung origin with an *IGF1R* non-frameshift insertion. Tumors were reclassified as ACC if they demonstrated areas with classic histologic features including a basaloid appearance, presence of cribriform architecture, and characteristic stroma (Fig. 2). 67% (2/3) of salivary tumors that possessed an *IGF1R* non-frameshift insertion and were submitted for CGP with a disease ontology of “salivary gland carcinoma, NOS” were either misclassified or underspecified ACC. Other potentially miscategorized cases with *IGF1R* hotspot alterations were originally classified as other salivary gland neoplasms, such as lacrimal duct carcinoma. After re-review, 100% (14/14) of salivary tumors with a hotspot *IGF1R* non-frameshift insertion had classic histologic features of ACC.

### **DISCUSSION**

This retrospective cohort study involving 326,911 samples provides the first pan-cancer genomic landscape of *IGF1R* alterations, including the identification of specific non-frameshift insertion hotspots. This analysis included primary tumors and metastatic lesions from all submitted subtypes of salivary gland tumors, including a total of 1912 non-ACC salivary gland tumors. A subset of the variants reported here have been observed in publicly available datasets, but the hotspots were not identified due to the rarity of the mutations<sup>16</sup>. These hotspot *IGF1R* alterations displayed notable disease specificity, occurring in ACC at a frequency 12.1-fold higher than any other cancer type. Interestingly, *IGF1R* hotspot alterations were not identified in any non-ACC salivary gland tumors. This specificity and partial mutual exclusivity with *MYB* fusions and *NOTCH* gene family alterations suggest a novel molecular subtype of ACC, defined by *IGF1R* non-frameshift insertions. Indeed, the specificity of *IGF1R* non-frameshift hotspots for ACC is high enough (100%, 14/14 cases

in this study) that in the context of a head and neck or salivary gland primary, identification of a mutation is suggestive of a diagnosis of ACC. This association may provide molecular support for the diagnosis in challenging cases.

IGF1R has recently been recognized as a critical component in an autocrine loop involving the *MYB-NFIB* fusion found in ACC<sup>11,12</sup>. Specifically, the *MYB-NFIB* fusion induces *IGF2*, which signals through IGF1R and induces cell proliferation via AKT-dependent signaling. Importantly, this cell proliferation is downregulated with IGF1R-inhibition. Our findings support and expand the critical role of IGF1R signaling in ACC pathogenesis by identifying, for the first time, that *IGF1R* alterations drive a subset of ACC which are negative for the canonical *MYB-NFIB* fusion. Additionally, IGF1R provides a potential therapeutic target in ACC for both the novel IGF1R-driven tumors and the classic *MYB-NFIB* driven cases.

Recent studies elucidating the cryo-EM structure of full-length *IGF1R-IGF1* complex in the active state provide a potential mechanistic basis for the two observed non-frameshift hotspots in ACC<sup>17</sup>. The smaller hotspot, insertions around amino acid position 663–666, corresponds to a linker region connecting two  $\alpha$ -CT motifs. Lengthening this linker reduced negative cooperativity and increased binding of IGF1 to IGF1R by ~50%<sup>17</sup>. The more frequent insertion hotspot, at amino acid position 1034–1049, occurs within the tyrosine kinase domain. These alterations likely operate in a similar fashion to oncogenic insertions described within kinase domains of other genes, such as *EGFR*, where they promote ligand-independent activation<sup>18</sup>.

Successful targeting of other tyrosine kinase hotspot non-frameshift alterations, such as in *EGFR* and *KIT*, highlights *IGF1R* hotspot insertions as a potential therapeutic target for investigational use of existing IGF1R inhibitors. Indeed, preclinical studies of figitumumab, lisitinib, and other IGF1R-directed therapies have demonstrated biological activity in a variety of tumor models that have activation of the IGF1R signaling axis<sup>19,20</sup>. Targeting the broad biomarker of upregulated IGF1R signaling has not translated to success in patients, where clinical trials of IGF1R inhibitors have produced disappointing results<sup>21,22</sup>. However, no studies have specifically selected for subjects with activating mutations of *IGF1R* itself, raising the possibility that existing inhibitors with demonstrated safety in humans could be repurposed for the rarer subset of patients with *IGF1R* hotspot insertions.

This study is limited by its retrospective observational nature, bias towards advanced stage disease, and a lack of functional confirmation for activated signaling downstream of the mutated *IGF1R*. Confirmation of pathologic diagnosis from outside institutions were unable to be performed for all cases. Furthermore, hotspot non-frameshift insertions may also constitute the key oncogenic drivers in non-ACC cancers, but additional conclusions were inhibited by small numbers and lack of functional data. However, the hypothesis of activated signaling is bolstered by the mouse studies and structural model. Additionally, the lack of RNA in the genomic profiling assay used presents a limitation of this study and may demonstrate an additional mechanism for MYB activation. Moreover, additional immunohistochemical analyses for IGF1R overexpression were unable to be performed given permission to perform additional testing for research purposes. Given the potential targetability of *IGF1R* kinase activation, additional studies are needed to further explore the functional activity and therapeutic response of *IGF1R* hotspot non-frameshift insertions.

## DATA AVAILABILITY

All data generated or analyzed during this study are included in this published article [and its supplementary information files].

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## AUTHOR CONTRIBUTIONS

M.M., J.L., B.D., and T.J. performed study concept and design; M.M., B.D., and T.J. performed development of methodology and writing, review and revision of the paper; M.M., B.D., and T.J. provided acquisition, analysis and interpretation of data,

and statistical analysis; J.L. provided technical and material support. All authors read and approved the final paper.

### COMPETING INTERESTS

The authors are employees of Foundation Medicine, Inc., and hold stock in Roche Holdings.

### ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was approved by the Institutional Review Board.

### ADDITIONAL INFORMATION

**Supplementary information** The online version contains supplementary material available at <https://doi.org/10.1038/s41379-022-01126-3>.

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